Rational Drug Design

Current Perceptions

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Indian National Science Academy
New Delhi

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Contents

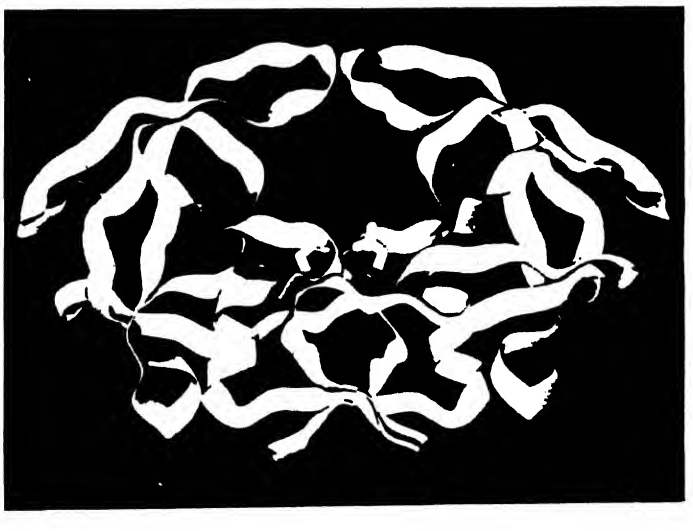
CHAPTER 1. RATIONAL DRUG DESIGN : CURRENT PERCEPTIONS

The collaboration between biologists and chemists have generated medicines of enormous benefit to society. The dynamics of this synergestic interaction combine the rigor of chemistry and the ability of the biologists to construct unifying concepts for diverse fields of investigation. The resulting contributions to the practice of medicine have reduced morbidity and mortality by increasing the quality of life. Building on the work of chemists like Robinson, Prelog, Pauling and Woodward, medicinal chemists have advanced rational drug discovery, particularly enzyme inhibitors, to a degree unthinkable even 20 years ago. Some of the major contributions to medicinal chemistry to human and animal welfare, stem directly from the creative application of, structural and electronic reasoning, advances in instrumentation and spectacular progress in molecular biology. However medicinal chemists have just begun to scratch the opportunities made possible by the impact of the double helix revolution of the fifties.

The most fundamental and lasting objective of synthesis is not production of new compounds, but production of properties.

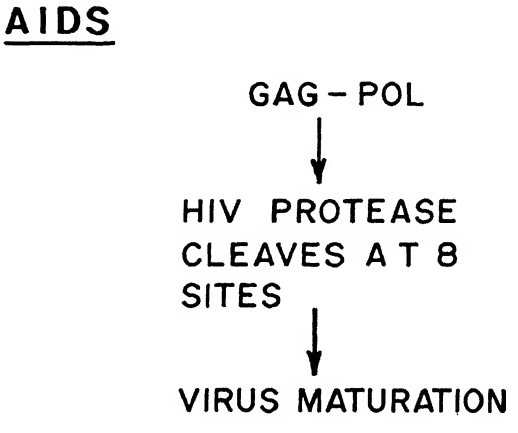
Much of the organic synthesis in the last three decades, rightfully, has been to understand the methodologies for making and breaking of covalent bonds. Recent developments in organic synthesis show increasing focus on the use of carbon frameworks to create desired properties, increasingly making use of non-covalent interactions.

Chart 1.1



In chart 1.1 , is presented HIV proteinase, an enzyme whose inhibition holds much promise in AIDS therapy. The enzyme breaks down a GAG-POL composite and if this is inhibited, the junctions remain the same and the AIDS virus cannot mature and proliferate. Fortunately, because of concerted efforts from various directions, a large amount of information relating to this enzyme is available. HIV proteinase [chart 1.1] has a perfect C_2 symmetry. It has 99 amino acid residues on one side and 99 on the other. The HIV proteinase cleaves the GAG-POL composite at eight sites. The scission is followed by virus maturation.[chart 1.2]

Chart 1.2

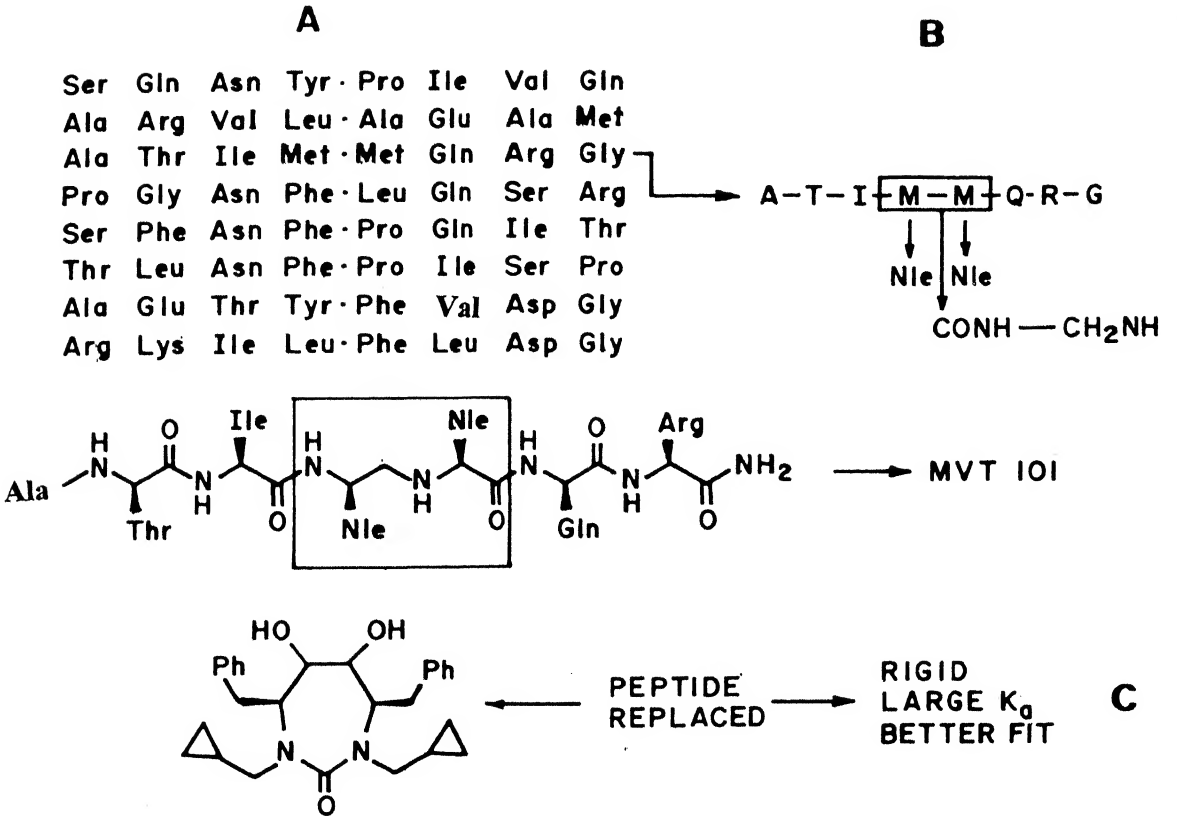


All the 8 cleavage sites have been identified.

Chart 1.3 refers to the scission points where inhibitors can bind to the central cleft. The HIV proteinase inhibitor, MVT 101, is based on scission site Met-Met in ATIMMQRE [chart 1. 3A, line 3] by replacement Met CONH Met → Nle-CH_2NH-Nle [chart 1.3.B]

The C_2 symmetry in HIV proteinase forms the focus of many HIV protease inhibitors. An example of a non-peptide C_2 symmetric, urea based inhibitor is presented in chart 1.3.C.

Chart 1.3



A novel development in the design of inhibitors is based on chiral reversal of the amino acid residues and directional reversal of the construct.

Because of the exclusive presence of L-amino acids in proteins, proteases can recognize and cleave only these sites. A desirable enzyme inhibitor is one where it is not only active but also resistant to the action of proteolytic enzymes. Indeed as a rule the half lives of small peptides *in vivo* is very short.

A recent approach to design inhibitors of CD4 receptors, a key protein in the immune system, is noteworthy, in the context of drug design. A 12 residue all L CD4 inhibitor was chosen to illustrate the strategy. The all L inhibitor is active but prone to proteolysis. The all D analog was resistant to proteolysis, but due to poor surface match, showed little activity. However the reverse D analog was both active and resistant.

The side chains of the L peptide and the retro-D-peptide have precisely the same side chain alignment, although their backbones are different. Thus, wherever recognition by the side chains by the receptor is the criteria, retro-D analogs of all L inhibitors can lead to desirable blockers. Conversely, the activity profile of a retro-D analog can provide clues of receptor recognition, either in terms of side chains (active) or arising from backbone (inactive) [chart 1.4]

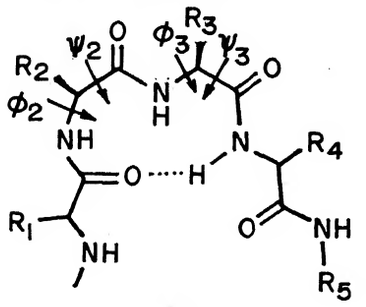
Chart 1.4

Inhibitors made of L-AA [NH_2-a^L-b^L-c^L....z^L-COOH] are prone to hydrolysis. Their D analogs [NH_2-a^D-b^D-c^D-....z-^{D}COOH], though stable are inactive. Because of the precise side chain match with L analog, the construct "RETRO-D" [NH_2-z^D.....c^D-b^D-a^D-COOH] can be active and stable to proteolysis, when side chain recognition is criteria for receptor contact.

β - Turn motifs [Chart 1.5 A] have provided a very excellent design for non-peptide inhibitors because they can provide the required spacer element by mimicking the Cα-Cβ bond vectors, which can be placed on a chosen scaffold [chart 1.5A]. Such turns have been generated by 10 - membered rings, bicyclic systems, benzodiazepines [chart 1.5B] steroids, glucose and several others.

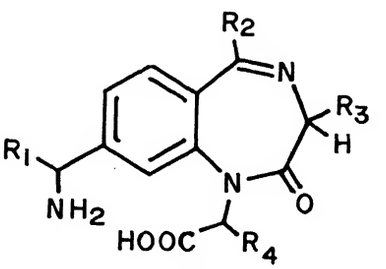
Chart 1.5

A) β- turn general structure



A

B) β- turn mimic using a benzodiazepine skeleton



B

Computer-based drug design has become a powerful tool in drug discovery. Protocols here have been quite successful in the identification of inhibitors. The discovery or design of structures that interact with target sites of known 3D surfaces, has led to rapid advances in "structure-based drug design". The progress made here will find applications in the domains of molecular recognition, material science and drug development.

General specifications are that the program must predict and come up with size, shape and electrostatic interactions. For example, if the nature of the inhibitor, either based on crystal structure or other means, is known, a versatile structural robot could be generated to provide, initially a large set of potential leads. Then with the help of a "sieving program", which can trim a side chain, remove or add groups etc., to come up with the best fit.

A number of software are available, notable ones being **DOCK, GRID, CAVEAT, FOUNDATION, CLIX.**

A brief description of programs largely in use are presented below:

MM3, CONCORD, WIZARD - Useful for 3-D coordinates of promising compounds

DOCK - The objective of molecular docking is to obtain lowest energy structures for the receptor - ligand complex. The method has discovered novel micromolar inhibitors for several receptors. Significant DOCK leads are presented below:

Affinities (μM)

System	Ist lead	End generation	Status
HIV proteinase	100	0.8	clinical trials
B DNA	10	------	----
Thymidylate-synthase	900	3	clinical trials
Hemaglutinin	100	5	----
CD_4	5	1	-----
Malaria protease	10	0.1	----

A notable success of DOCK is the discovery of the nonpeptide HIV proteinase inhibitor haloperidol (K_i 100 μM) whose thioketal (K_i 1.5 μM) has been co-crystallized with HIV proteinase and structure elucidated by X-ray.

GRID Analyses the active site and is useful for the design of more potent inhibitors based on existing ones. The program has been successful in the identification of potent inhibitors of enzyme associated with the release of sialic acid from the influenza virus, a critical step in the viral cycle.

MONTE CARLO Energy minimization program has found much use in the design of inhibitors of purine nucleoside phosphorylase (PNP), now in clinical trials.

CAVEAT focuses on templates as starting points for chemical modification

FOUNDATION can combine models of crucial ligand atoms.

CLIX uses receptor site features to define possible binding configurations.

GROW identifies regions of high complementarity, by docking functional groups, independently, to receptor.

Leads, based on some idea of active sites, that used to take 2-3 years, by conventional procedures can be done, with much more prospects, in a matter of hours by computer methodologies.

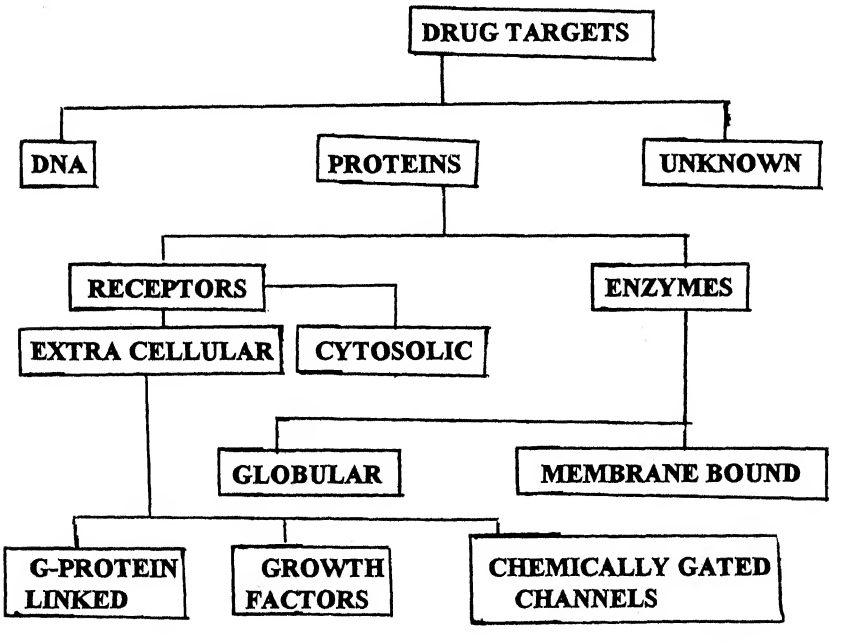
The top drugs of 1992 are presented in Table 1. A careful reading would show that the drugs profile reflect societal concerns of the period, with focus on cardiovascular, antibacterial, allergy abatement, ulcer alleviation and antidepressant therapies. In the coming decade, this focus would be on drugs and agents having effect on the central nervous system.

Chart 1.6 illustrates how to reach drug targets. A target drug may be related to DNA or proteins or an unknown system. In the realm of proteins, it may be related to receptors or enzymes. The receptor could be extracellular or cytosolic. The extracellular receptors may be G-protein linked or related to growth factors or chemically gated ion channels.

Table 1. Top drugs - 1992

Drug	Activity [Utility]
Ranitidine	H_2 Antagonist [anti ulcer]
Captopril	ACE inhibitor [anti hypertensive]
Enalpril	ACE inhibitor [anti hypertensive]
Nifedipine	Calcium channel blocker [anti angina]
Atenolol	β Blocker [anti hypertensive, anti angina]
Cimetidine	H_2 Antagonist [anti ulcer]
Dielefene Sodium	Cyclooxygenaseinhibitor [anti inflammatory]
Cefaclor	Cephalosporin [antibacterial]
Ciprofloxacin	DNA gyrase inhibitor [antibacterial]
Albuterol	Antiasthamatic
Fluoxetine	5HT uptake inhibitor, anti depressant
Lowestatin	HMG CoA inhibitor, anti hypercholesterol
Diltiazem	calcium channel blocker, anti anginal
Clavulanic acid	β-lactamase inhibitor
Acyclovir	DNA polymerase inhibitor; anti herpetic
Naproxen	PG synthesis inhibitor, anti inflammatory
Terfenadine	H_1 receptor antagonist, antihistamine
Ceftriaxone	β lactum antibiotic
Piroxicam	non steroidal anti inflammatory
Omeprazole	proton pump inhibitor, anti ulcer

Chart 1.6



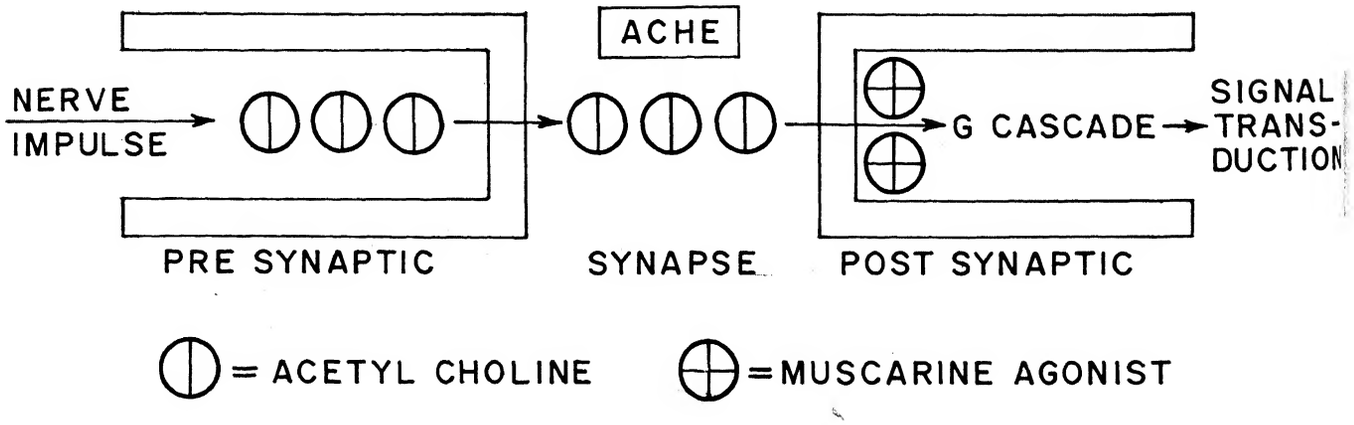
Receptors as drug targets for drug design has produced spectacular results. In general, the receptors belong to 4 super families:

1. G-proteins [adrenergic, muscarine, cholinergic, dopaminergic, serctonergic, several peptidergic, angiotensin, tachikinins].
2. Growth factor receptors [for prolactin, insulin].
3. Chemically gated channels [nicotinic, cholinergic, GABA].
4. Cytosolic receptors for steroid and thyroid hormones.

Progress relating to muscarine-acetyl choline receptor system is particularly illustrative.

Alzheimer disease (AD) arises from selective degeneration of cholinergic neurons. On nerve impulse, acetyl choline is released from the presynaptic fibre, through synapse to the post synaptic fibre where, by a G cascade, resulting in signal transduction. Accentuation of the cholinergic transmission has been probed by (i) inhibition of acetylcholine esterase [ACHE] to potentiate effects of endogenous neurotransmitter and (ii) placement , at post synaptic sites, muscarine agonists , which promote the G cascade [chart 1.7]

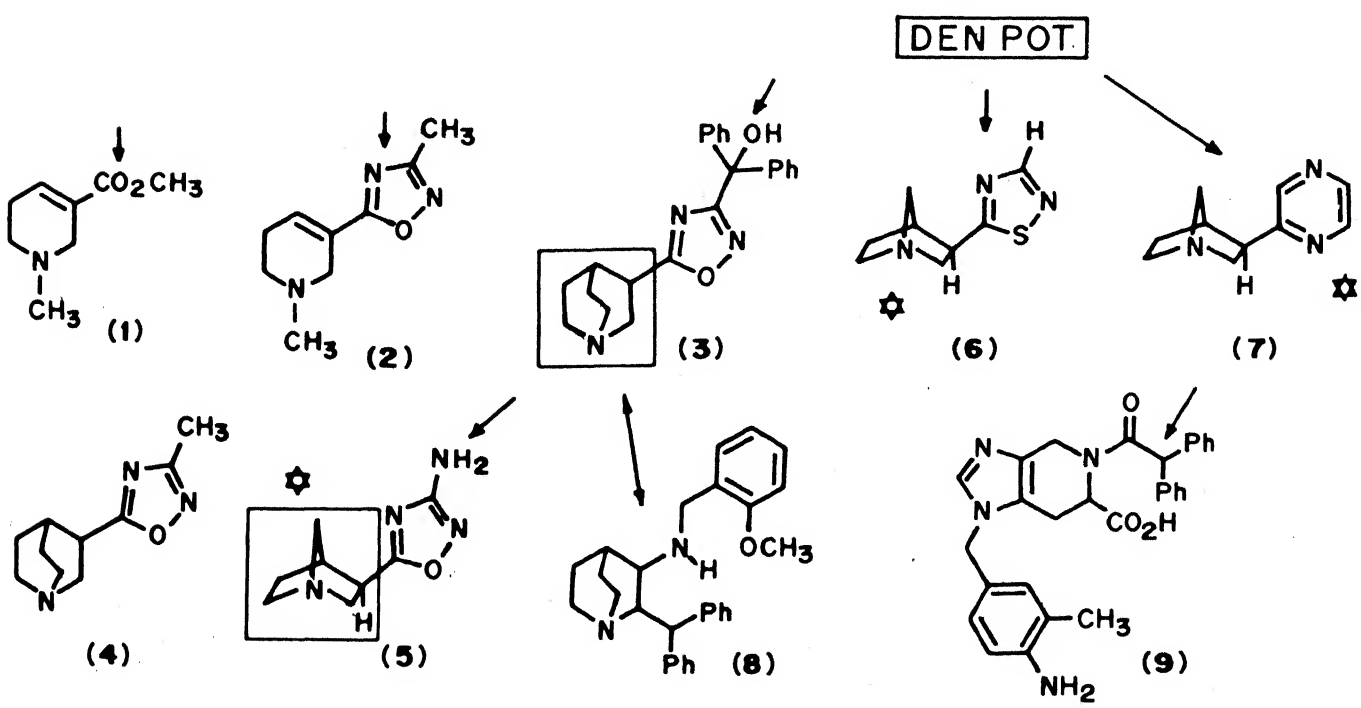
Chart 1.7



The rational modification of the partial muscarine agonist, arecoline (1) [chart 1.8] to full agonists and antagonists would be a good illustration of drug design. Computer modelling enabled the replacement of the susceptible COOMe in (1) to oxadiazole (2) which was only moderatley active. Muscarine agonists are believed to exist in the protonated form at receptor sites. Calculations with focus on charge distribution suggested choice for spherically symmetrical charge distribution. This coupled with the notion that further hydrogen bonding possibilities around the oxadiazole would be beneficial, led to discovery of partial agonist (4), full agonist (5), and antagonist (3). Electrostatic potential energy mapping with DENPOT led to the alternate heterocyclic systems 6 and 7 [Chart 1.8]

Compound (3) is a muscarine antagonist, because of the hydrophobic ligand to the oxadiazole. A factor of help with respect to design of receptor site ligands is that one receptor may be used to design ligands for another receptor. Thus (8) and (9) are antagonists for neurokinin peptide receptor (NK1) and angiotensin receptor respectively [chart 1.8].

Chart 1.8

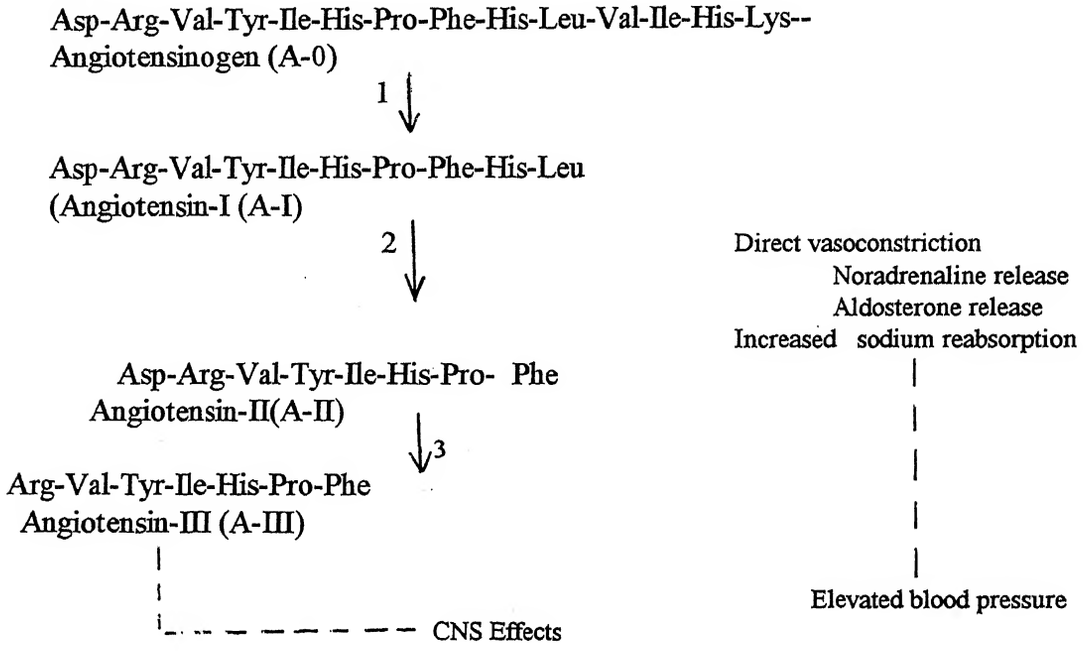


Agonists are hydrophilic , antagonists are lipophilic , ✡ Active compound

The renin - angiotensin system (RAS), [chart 1.9], is responsible for the production of the hormones angiotensin-II (A-II) and A-III ,which are essential for maintenance of normal blood pressure.

Chart 1.9

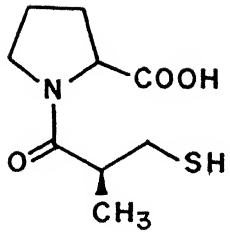
RENIN - ANGIOTENSIN SYSTEM (RAS)



The renin-angiotensin system. Interventions that prevent the production of A-II will reduce elevated blood pressure by the mechanisms shown. 1, renin; 2, angiotensin converting enzyme; 3, aminopeptidases.

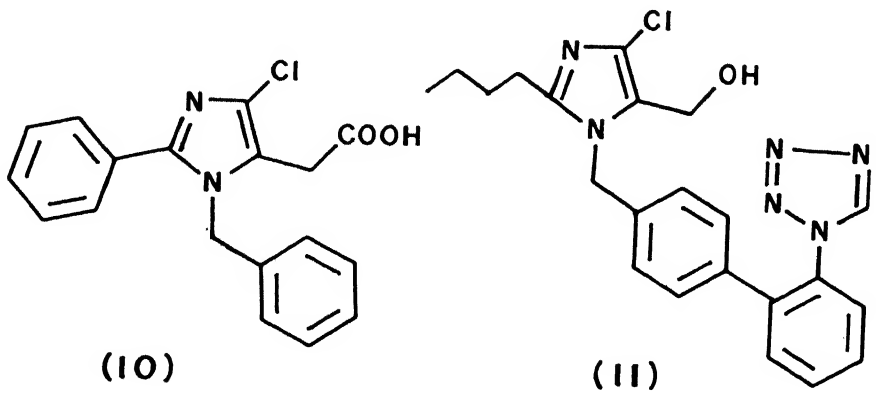
Blood pressure control has been most successfully achieved either by prevention of the formation of the potent vaso constricter A-II or by blocking of the action of A-II by superior antagonists. The first has been achieved by potent angiotensin converting enzyme (ACE) inhibitors.

Captopril, now a billion dollar product is a superb example of rational drug design using mechanistic and structural information available for an homology enzyme (carboxy peptidase A). Captopril is a potent ACE inhibitor.



captopril

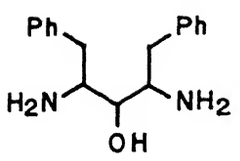
Search for A-II antagonists, that will block the acceptance of A-II by receptors, have given valuable drugs. Indeed, from the lead compound (10) through conventional screening, progressive design has resulted in (11) which is now undergoing human trials, orally as a A-II blocker.

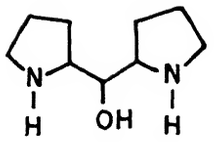


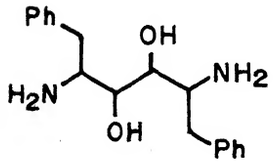
Design of enzyme inhibitors have been earlier described for the C_2 symmetric HIV proteinase by group replacement. The notion that the active site configuration of HIV proteinase also would have C_2 symmetry has led to several active inhibitors where a C_2 symmetry could be seen in most cases [Chart 1.10].

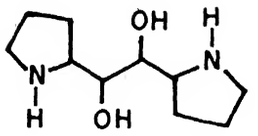
The drug prospect A74704 [Chart 1.10] has been cocrystallised with HIV proteinase. X-ray of the composite shows that 2 units of A74704 are precisely arranged around the C_2 symmetry axis of the enzyme [chart 1.11]. This set of experiments have shown possible correlation of symmetry elements present at the receptor site with compounds that can match this symmetry and which are likely to generate inhibitors.

Chart 1.10









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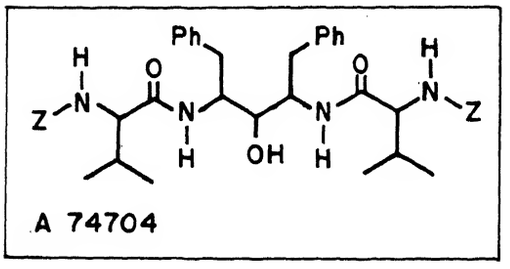

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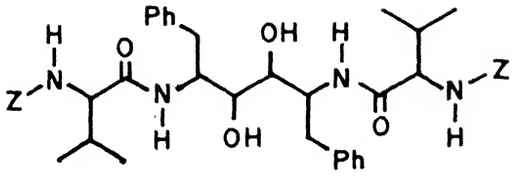
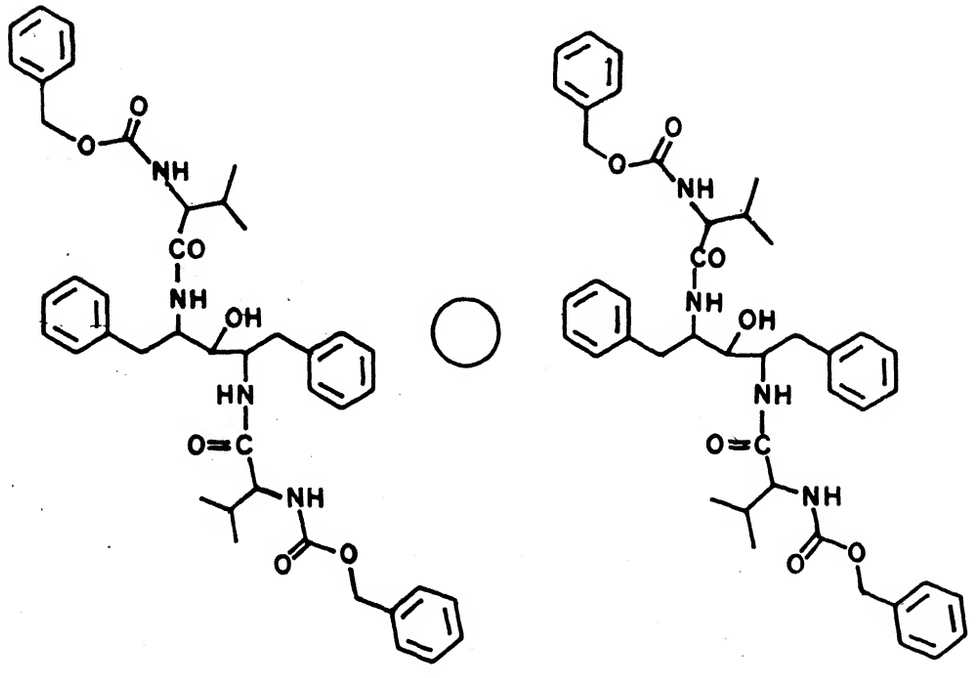


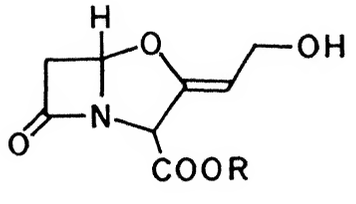
(a)3R,4R; (b)3S,4S;(c)3R,4S

Chart 1.11

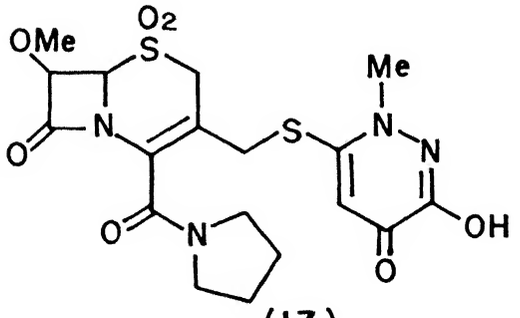


The degeneration of soft and connective tissues, a syndrome associated with hereditary diseases and viral infection, is often associated with high elastase activity. This enzyme degrades elastin leading to grave pathological problems. The design of elastase inhibitors is yet another success in drug design.

The discovery of elastase inhibitor activity of β–Lactamase, clavulanic acid (12), led to rapid development of potent elastase inhibitor (13) which is in pre-clinical trials.



(12)



(13)

Rational drug design, in areas of societal concern, has been covered. This subjective selection should reflect the best in such endeavours. Hopefully this account has provided an overview of exciting horizons in the domain of drug discovery. The methodologies presented denote a radical departure from slow conventional methods to rapid, innovative, multidiscipline-based protocols.

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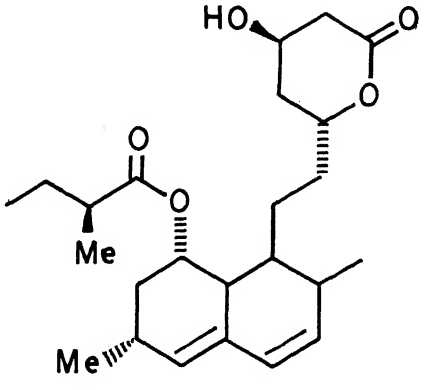
2. MEDICINAL CHEMISTRY IN THE GOLDEN AGE OF BIOLOGY

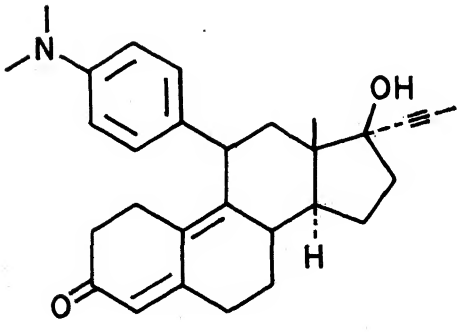
Almost all drug design strategies outlined in the first chapter are rooted in our expertise in making and breaking bonds, thus enabling the creation , by organic synthesis, practically any type of structures. This aspect is the focus of the present chapter.

The blocking of cholesterol biosynthesis is the goal pertaining to several ailments, the most notable one being that relating to the cardiovascular system. The understanding of cholesterol biosynthesis from acetate has led to the potent drug, lovastatin (1) which inhibits cholesterol biosynthesis.



(1)

Mifepristone (2), an abortifacient in conjunction with PG and proscar (3), for treatment of enlarged prostate , are results of design, based on a large number of leads.



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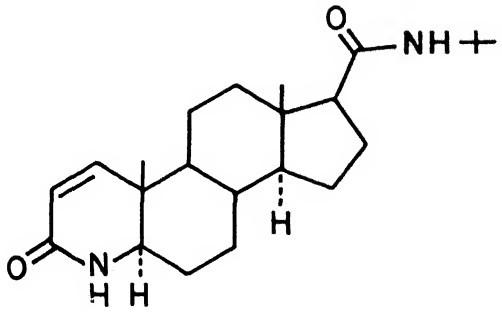


(3)

The developments in organic chemistry, in terms of structures and concepts ,have led to great discoveries. The notion that agonists bind tightly to the receptor in the activated conformation, whereas antagonists bind to receptors tightly in the ground state conformation, coupled with a further understanding of non-covalent interaction between the ligand and receptor has led to the design of numerous active compounds.

At one time small peptides prevailed largely in the organic chemistry domain. This view underwent a radical change with the discovery of hypothalamic hormones (4), (5), (6) and enkephalins (7) and (8) which are the endogenous ligands for the morphine receptor.

The bioresponse profile of (5) made it a suitable candidate against juvenile diabetes, provided its *in vivo* stability can be enhanced. The success here forms a good example in drug design. The β- turn motif (9) present in (5)-(10) (chart 1) became the focus for imparting *in vivo* stability.

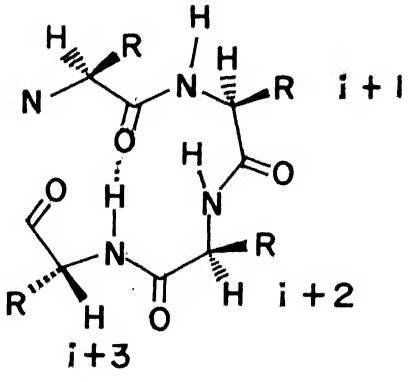
Pyro–D–H–W–S–Y–G–L–R–P–G–NH_2 (4)

A–G–C–K–N–F–F–W–K–T–F–T–S–C–OH (5)

Pyro – H– P– NH_2 (6)

Y–G–G–F–M (7)

Y–G–G–F–L (8)



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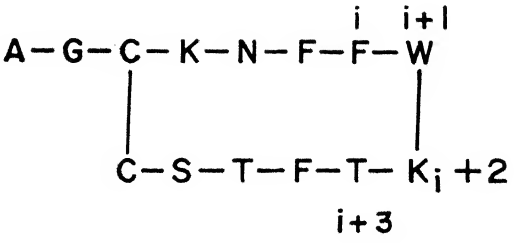
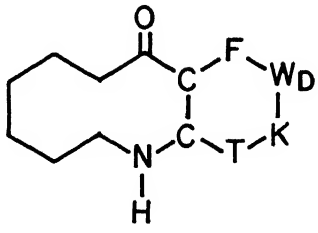
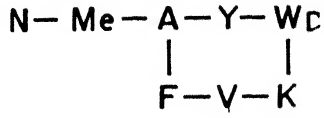


(10)

Replacement of W_8 of (10) with the β-turn stabilizer W_D^8 ,deletion of spatially proximate K^4, N^5, F^6 and F^{11}, T^{12}, S^{13} and linking the terminii afforded the stable and active (11). Further modelling gave (12), which although having only 2 of the 14 amino acids of the natural (10) is 50-100 times more potent.

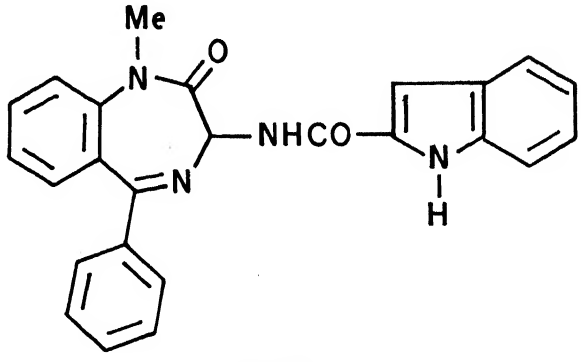


(11)

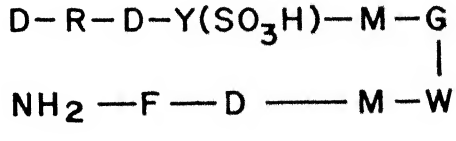


(12)

As stated previously, the design and synthesis of nonpeptidic ligands to replace naturally occurring, active, but short half-life peptides, is a fertile area for drug discovery. The flexible limits here become obvious when realized that the rationally designed, orally active (13) is as potent as the peptide (14) as an antagonist of cholecystokinin (CKK)

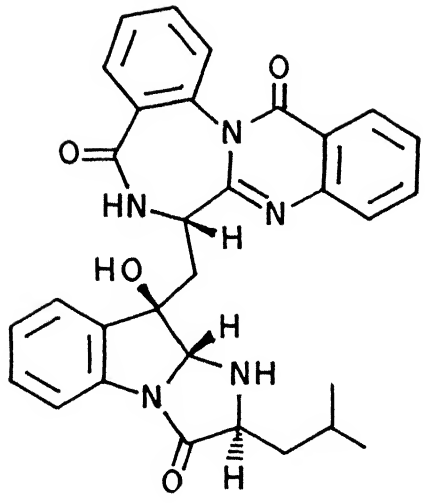


(13)



(14)

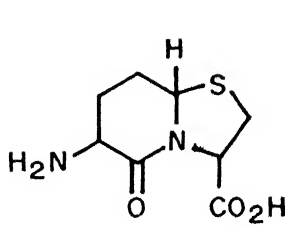
Peptidomimetics research owes its impetus to the desire to discover non-peptides that bind to peptide receptors, but which have enhanced bio-availability. Non-peptides that have peptide bonds, have been screened and tested and have led to important lead compounds notable amongst which is the unusual (15).

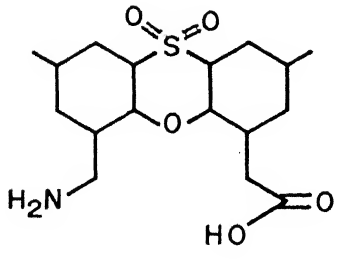


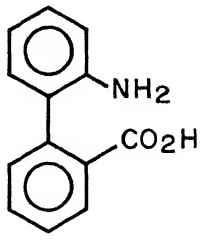
(15)

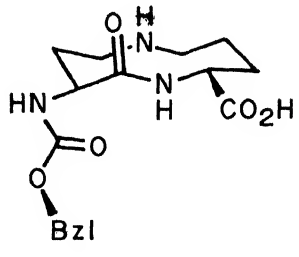
The ingenuity of organic chemists to conceive and construct β- turn mimics can be seen in representative examples presented in Chart 2.1, many of which exhibit notable activity.

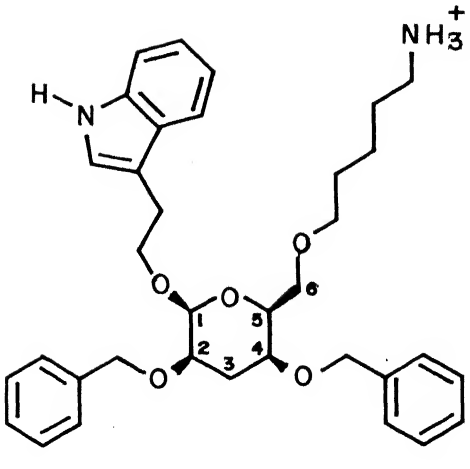
Chart 2.1

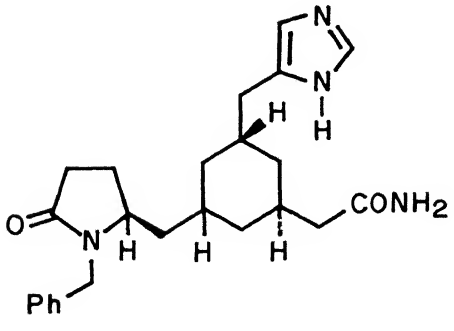


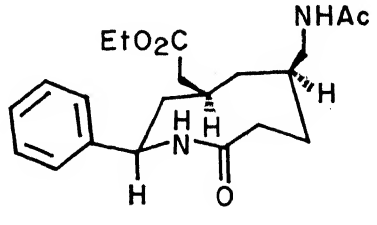


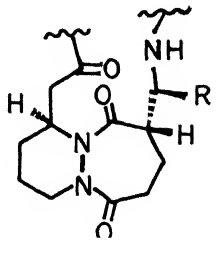




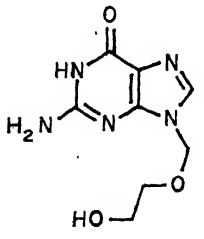




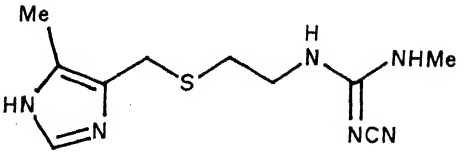




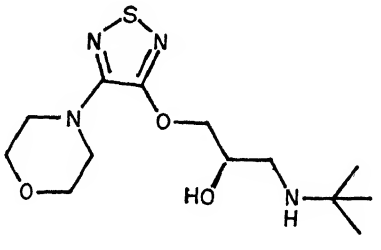
A great asset of organic chemists is the "feel" they have for compounds of medicinal interest, arising from, knowledge of metabolic pathways, possible structural interactions at ligand-receptor sites and reactivity profile correlations. This is attested with synthesis of a large number of unusual compounds having excellent drug profile, cimetidine, acyclovir and blocadrin being three excellent illustrations:



ACYCLOVIR ANTI HERPES



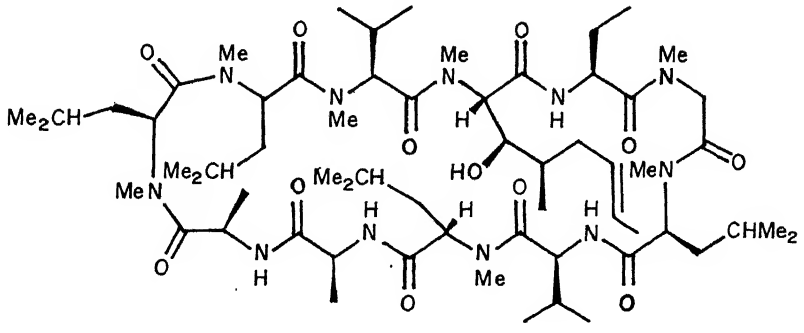
CIMETIDINE FOR ULCERS



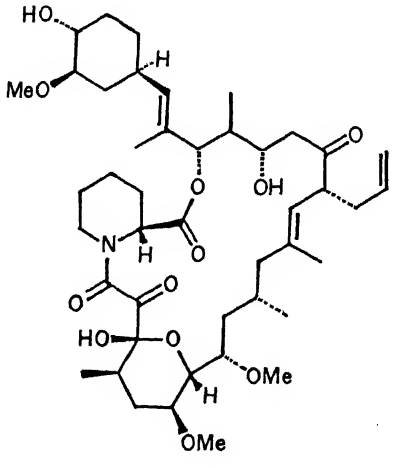
BLOCADREN FOR SAFE AND TOPICAL FOR GLAUCOMA

Organic chemistry in the realm of drug discovery continues to be the backbone of therapeutics. With all the advances in this and related disciplines, it would still be hopeless to design an entirely novel structural system targeted to a specific action. Conversely given a new structure, its biological profile would indeed be hard to predict.

Thus, surprises in natural products research will continue as has been with the immunosuppresants cyclosporin (**16**), FK506 (**17**), the anti-cancer agents ene diyne antibiotics and taxol. The thrill of discovery will be there and the quest to secure new structures and establish their biological profile will continue for a long time to come.



(16)



(17)

Leading References

1. Medicinal chemistry in the golden age of biology. Hirschmann. Angew. chem. Int. Ed. Engl., **30**, 1278 (1991).

3.DRUG DESIGN BASED ON PROTEIN STRUCTURES

The focus of this chapter is the design of drugs based on protein/peptide structures and on the chemical reactivity at target sites.

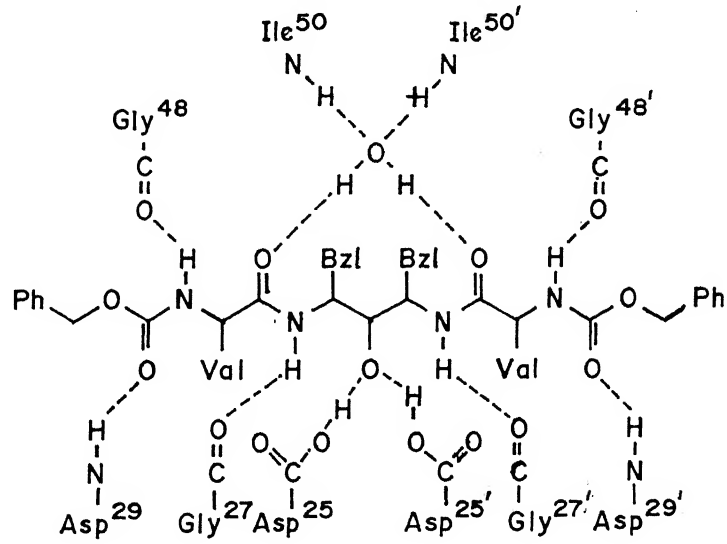
The availability of bioactive proteins/peptides is critical to drug design. Whilst a great many functional proteins are available in sufficient quantities, critical proteins and many bioactive peptides were hitherto available in only ~ μg amounts. The protocols for protein/peptide amplification, shown below,, undergoing increasing sophistication, will make available any protein/peptide.

Protein/peptide [~μ g] → Sequence → gene synthesis
PCR amplification → cloning → expression (mgs)
structure determination by X-ray/solution studies.

The peptide/protein can be subjected to protocols related to drug design. This will be illustrated with selected examples.

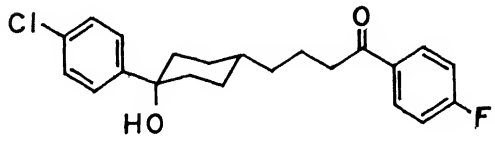
The crystal structure of HIV proteinase coupled with knowledge of the precise active site surface has enabled the design of a number of inhibitors based largely on the profile of hydrogen bonding between the ligand and the receptor.

The positioning of HIV proteinase inhibitor A74704 at the active site [Chart 1.11] and its hydrogen bonding profile is found exactly as predicted[see below]. Of even greater benefit is the successful design of non-peptide inhibitors, using only the enzyme active site as the starting point.

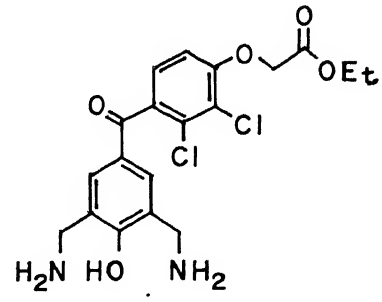


DOCK generated a 3D template of key potential binding sites, mapping of which to a structural data base led to the inhibitor haloperidol (1).

The key hydrogen bonding interactions shown above has been used to generate a 3D pattern of atoms. Using this information a data base search of small molecules with ALADDIN ,led to inhibitor (2).



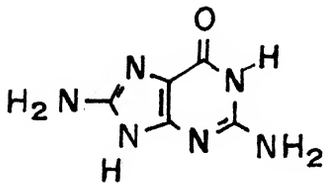
(1)



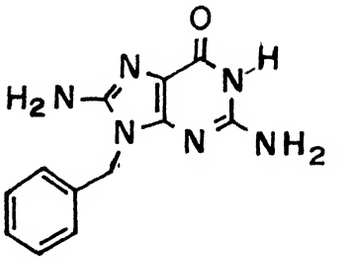
(2)

Data of active site alone may be insufficient, since the geometry here changes substantially on ligand binding. Thus, in such cases coordinates of ligand-active site composites are needed from X-ray data. This aspect can be illustrated with purine nucleoside phosphorylase (PNP), whose inhibition may find use as T-cell selective immune suppressants in the prevention of organ transplant rejection and certain types of cancers.

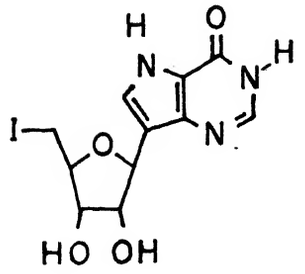
As the first step in the rational design of PNP inhibitors, X-ray data, as bound ligands, were secured for the following PNP inhibitors (3-6).



(3)



(4)



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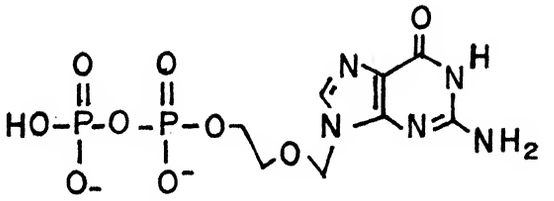


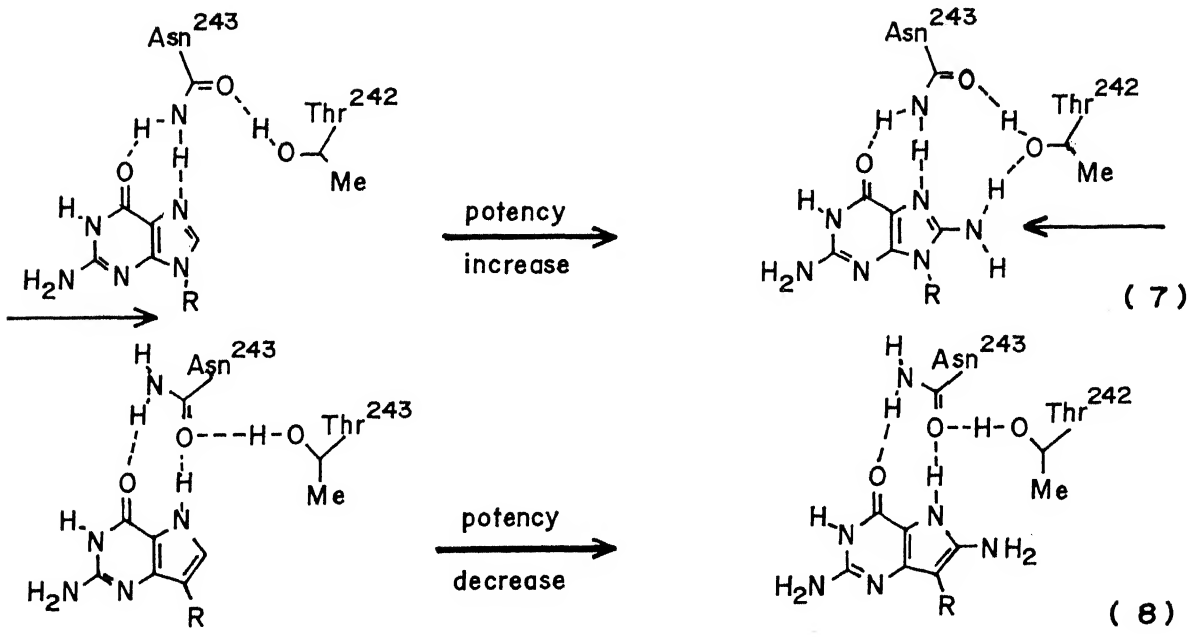
(6)

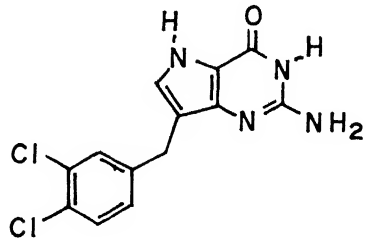
The co-crystal data was able to rationalize the effect of ligand substitution.

A hydrophobic group at 9 position in (4) enhances the activity arising from favourable interaction with the hydrophobic region of the ribose binding site. The potency enhancing effect of introduction of amino group at 8 position in (7) arises from additional hydrogen bond made with Thr 242.

However, the potency is decreased with the 9-de aza system (8), because of an 2.5 A° shift required for the hydrogen bonding with Asn 243, the additional hydrogen bonding with Thr 242 becomes not possible.

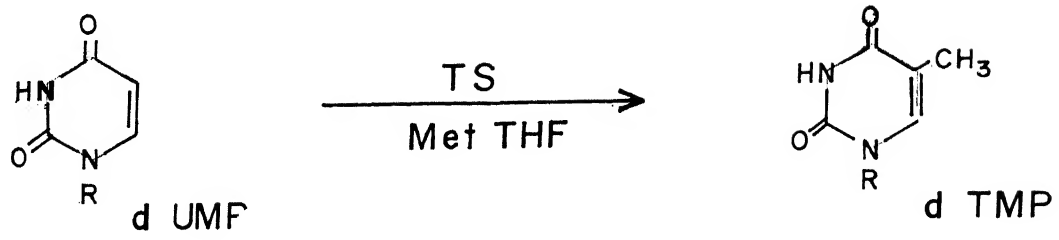
These considerations have lead to the identification of (9) as an *in vivo* PNP inhibitor



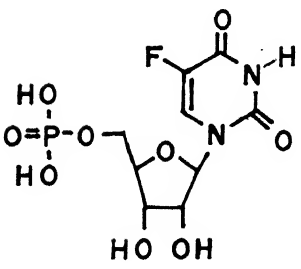


(9)

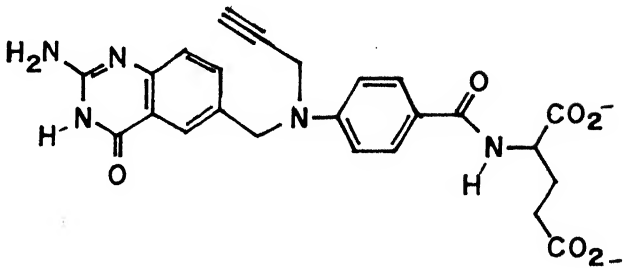
Ironically, there is a single pathway for the uracil (dUMP) → Thymidine (dTMP) change. Thymidilate synthetase (TS) mediates this reaction, which involves "CH_2O" transfer from Met THF and reduction.



Inhibitors of TS has been considered as agents for preventing cancer cell proliferation. Before structural information of TS was available, inhibitor design was based on knowledge of substrate, co-factor and reaction intermediates. These included substrate analogs such as (10) and FH_4 antagonists, eg. (11).



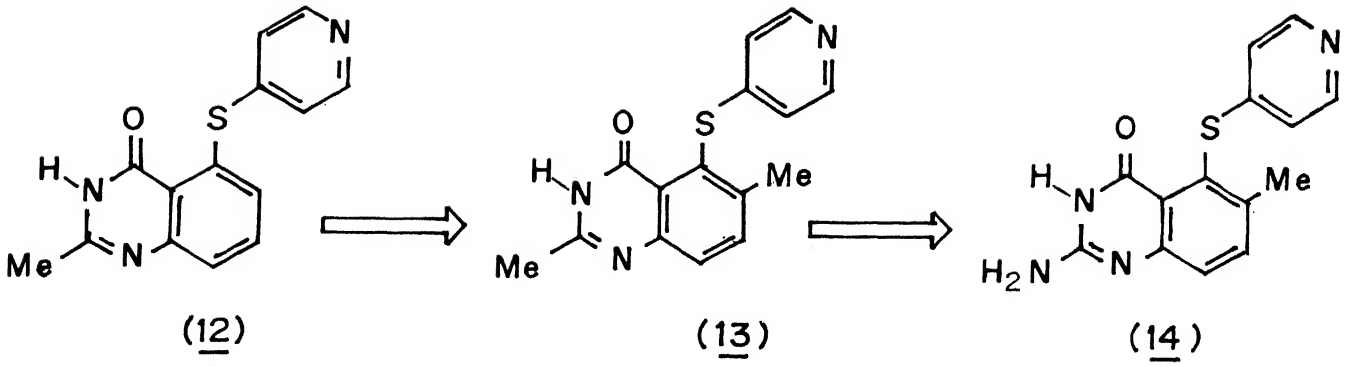
(10)



(11)

The subsequent availability of the X-ray structure of TS and ternary co-crystals of dUMP and (10) with the folate antagonist (11), structure based design became a reality, thus providing an excellent illustration of the best in drug design.

3D-Surfaces generated from X-ray provided the first lead compound (12). The crystal structure of this compound, determined in a ternary complex with TS and FdUMP suggested (13), having an additional methyl group, a better inhibitor. The compound was prepared and showed the predicted enhanced potency. Structural analysis of (13) when bound in a ternary complex [TS + FdUMP] predicted a further enhancement of inhibitor activity in (14), which was experimentally confirmed. Thus, two small changes on lead (12) increased the potency 200-folds. Compound (14) is in clinical development as an anti-cancer agent .

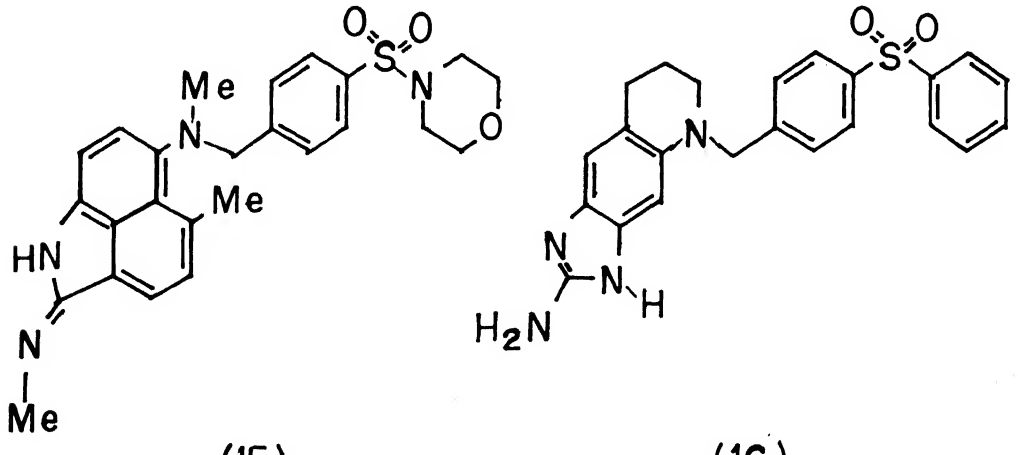


(12) (13) (14)

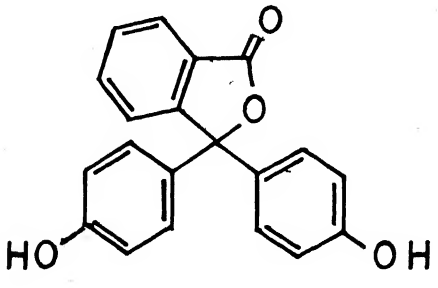
Relative potency
vs. E. coli TS

1 20 200

Another approach here is to "remove" (11) from the ternary complex and design inhibitors based on surface available in the complexed state. In this manner GRID provided the two inhibitors (15) and (16). Having an idea of the active site and using a weak lead, a search of Aldrich catalog identified phenolphthalein (17), by DOCK, as a TS inhibitor!



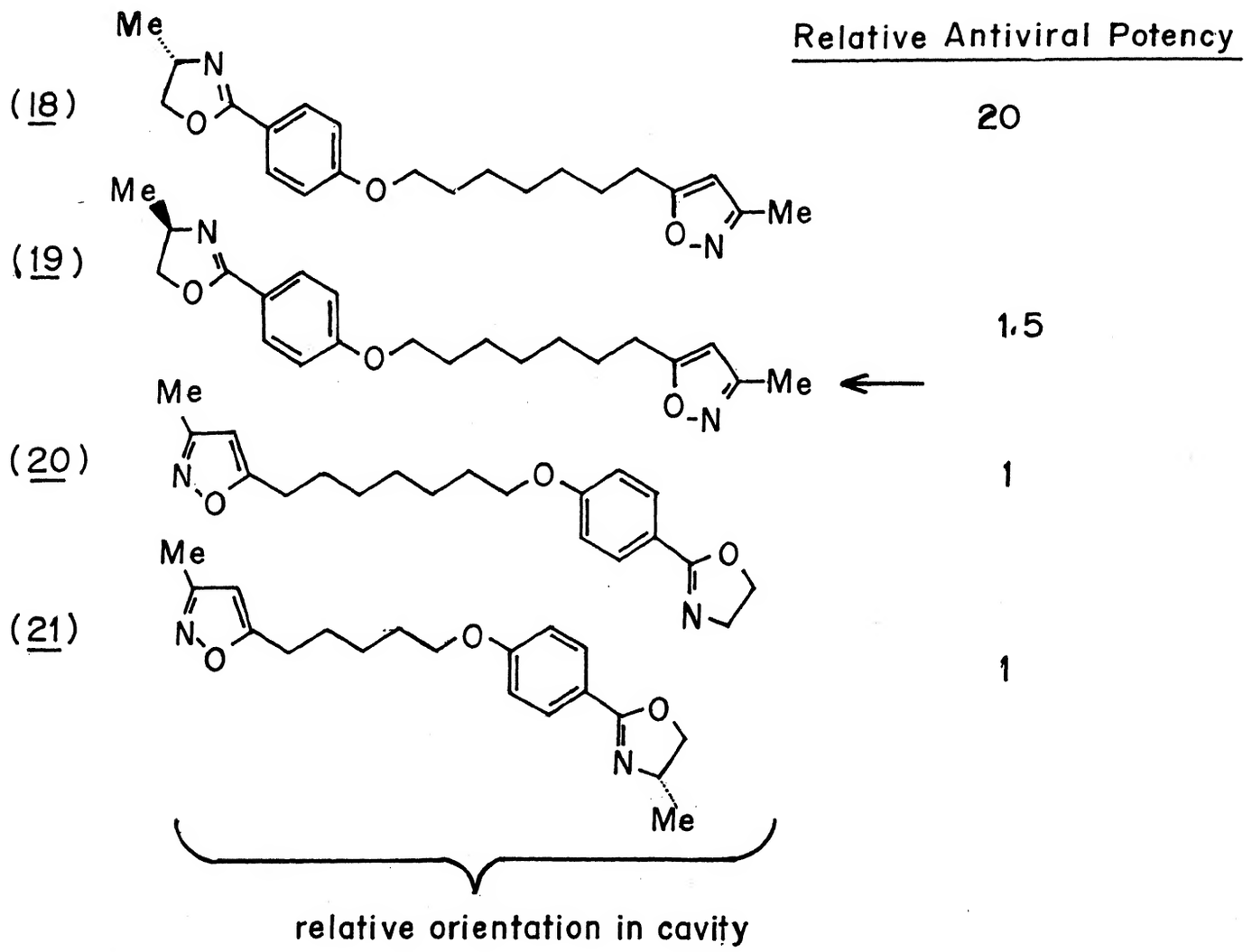
(15) (16)



(17)

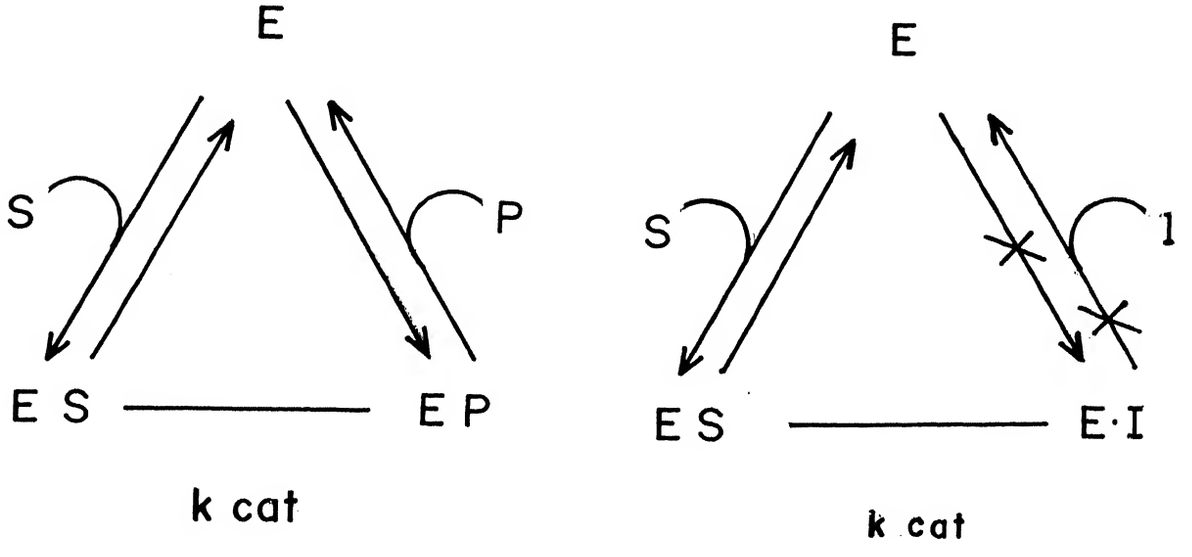
Rhino viruses (+ strand RNA) are the major cause for common cold. Screening resulted in (**18**), which prevents the removal of the RNA protein coat, after entering the host. Thus RNA is not exposed and viral cycle is stopped.

The crystal structure of human rhino virus (HRV-14) and that complexed with (**18**) showed that the inhibitor binds in a hydrophobic cavity, under the canyon on the surface. Interestingly, the chirality of the oxazoline methyl in (**18**) is important, because of interaction with Leu 106 and Ser 107 on the surface of the canyon. This, and consequences of other structural changes on the inhibitor activity, is presented below.



Mechanism based inactivation of target enzymes is a fertile domain for drug development. It has also intellectual appeal in the sense that it incorporates facets of chemistry, biology and medicine.

A fatal kinetic attraction of the enzyme active site to a carefully structured inhibitor either whole or in part makes the enzyme-inhibitor [whole or part] bond permanent thus breaking the catalytic cycle .



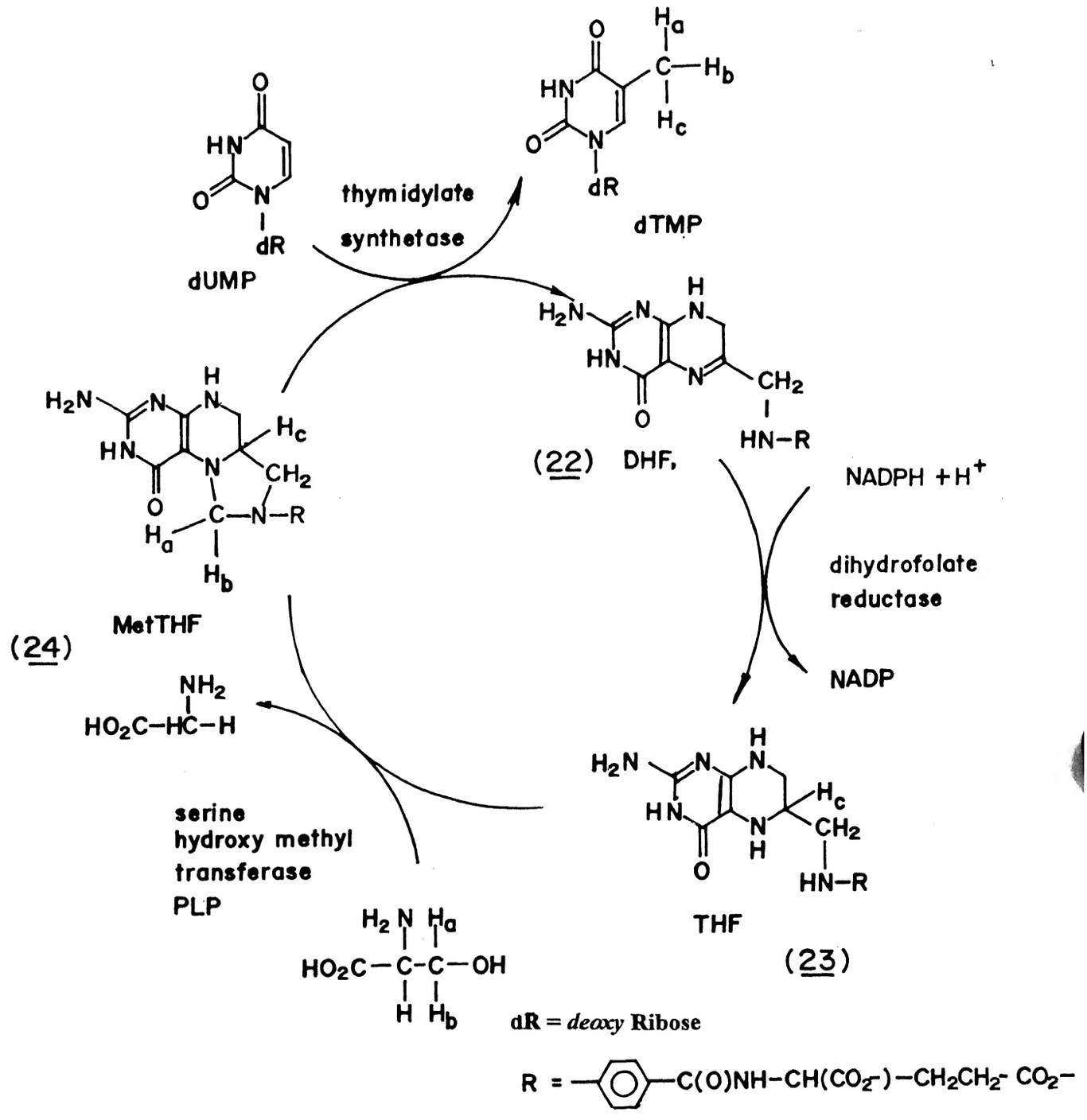
Enzymes, with potential in drug development, by mechanism based inactivation are presented in Table 3.1. The strategy for this approach can be illustrated with few examples.

As stated previously, the dUMP → dTMP change involves methylation, where one carbon is provided by Met THF. TS brings about this change. Two additional enzymes, dihydro folate reductase (DFR) and serine hydroxymethyl transferase (SHMT), regenerate

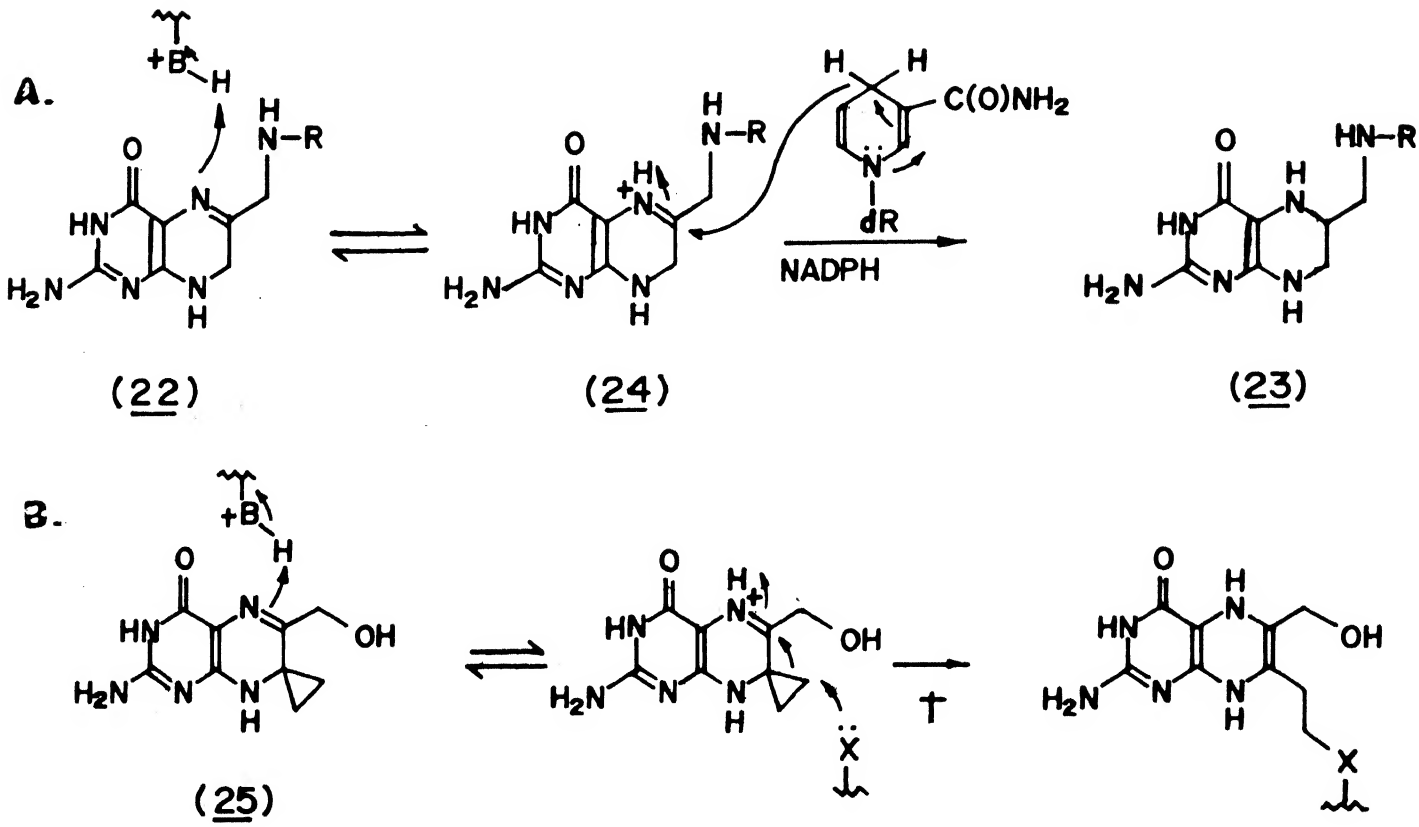
Table 3.1

Enzyme	Therapeutic goal
S-adenosyl homocysteine hydrolase	antiviral agent
alanine racemase	antibacterial agent
D-amino acid amino transferase	antibacterial agent
γ- aminobutyricacid amino transferase	anticonvulsant agent
arginine decarboxylase	antibacterial agent
aromatase	anti cancer agent
L-aromatic amino acid decarboxylase	synergestic with anti Parkinsonian drug
dihydrofolate reductase	anti cancer; anti bacterial&anti protozoal
dihydro orotate dehydrogenase	anti parasitic and anti cancer agent
DNA polymerase I	anti viral
dopamine β -hydroxylase	antihypertensive;pheochromo,cytoma agent
histidine decarboxylase	anti histamine and anti ulcer agent
β– lactamase	synergestic with antibiotics
mono amine oxydase	anti depressant,anti hypertensive and anti Parkinsonian agent
ornithine decarboxylase	anti cancer and anti protozoal agent
serine protease	anti inflammatory; emphysema, respiratory, digestive and degenerative skin disorders; anti coagulant and anti viral agent
testosterone 5-α reductase	anti cancer agent
thymidilate synthetase	anti cancer agent
xanthine oxidase	uricosuric agent
thyroid peroxidase	anti thyroid agent

Met THF to complete the cycle . In principle, blocking of either TS or DFR or SHMT will block DNA synthesis, thereby causing cell death. In practice, inactivation of TS and DFR are targetted in cancer therapy. The drug methotrexate is a DFR inhibitor, by a competitive mode. The salient features of this vital cycle are illustrated below.

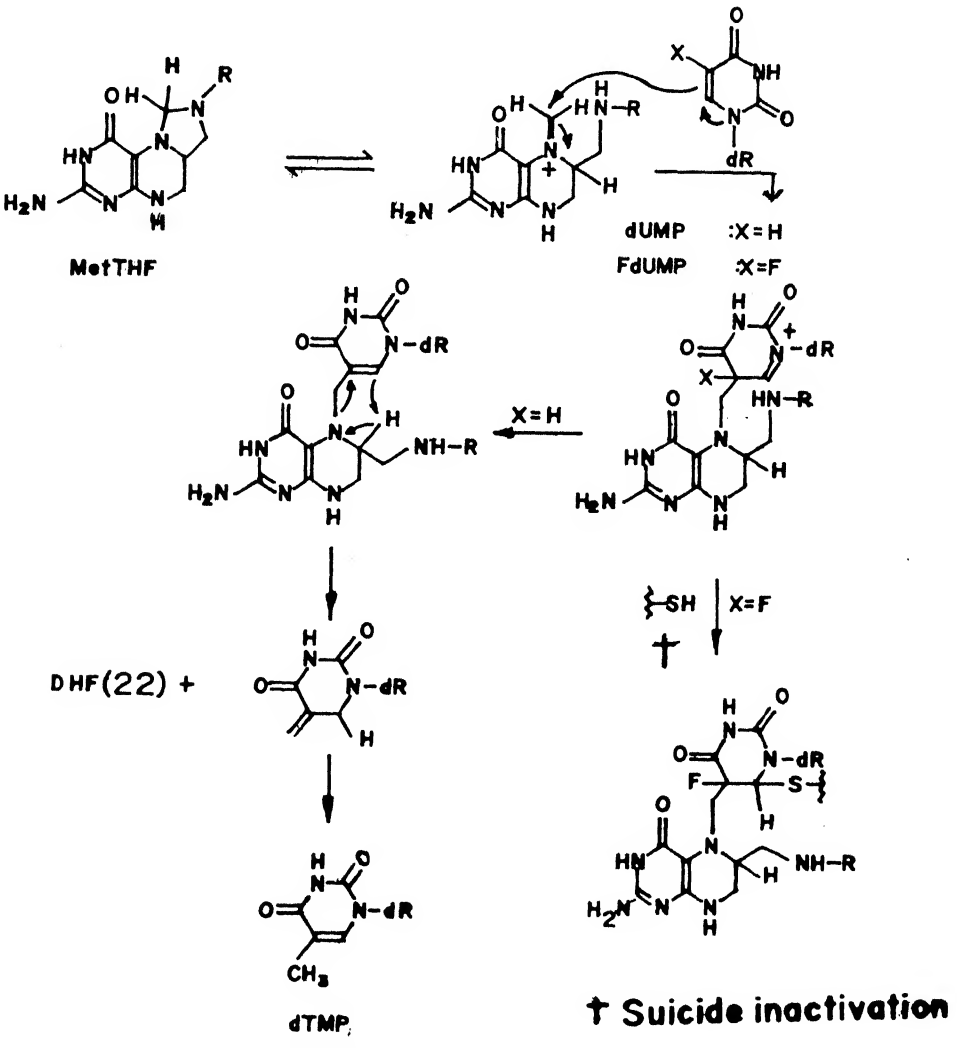


In the following, Path A re resents the normal (22) → (23) change by DFR. One could at once see from Path B, how the enzyme inactivation takes place when, instead of (22), compound (25) is used.



Enzyme; † Suicide inactivation step

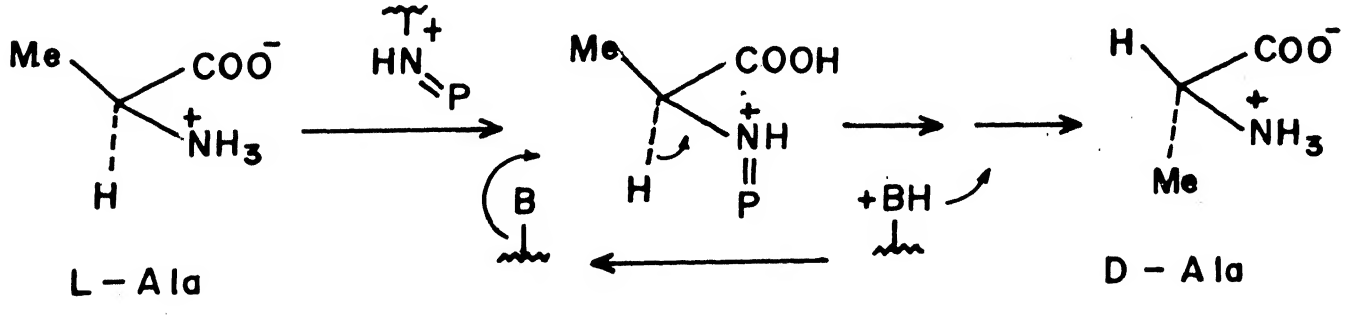
In size, F and H are about the same. The inhibition of dUMP → dTMP change by 5-fluoro uracil arises from inability to undergo a key prototropic shift to neutralize an electrophilic intermediate. Kinetically an SH group in the enzyme steps in, thus destroying itself!



Having outlined the salient features, mechanism based inactivation of some key enzymes are illustrated below, which shows the normal enzyme mode and inhibition based on enzyme participation.

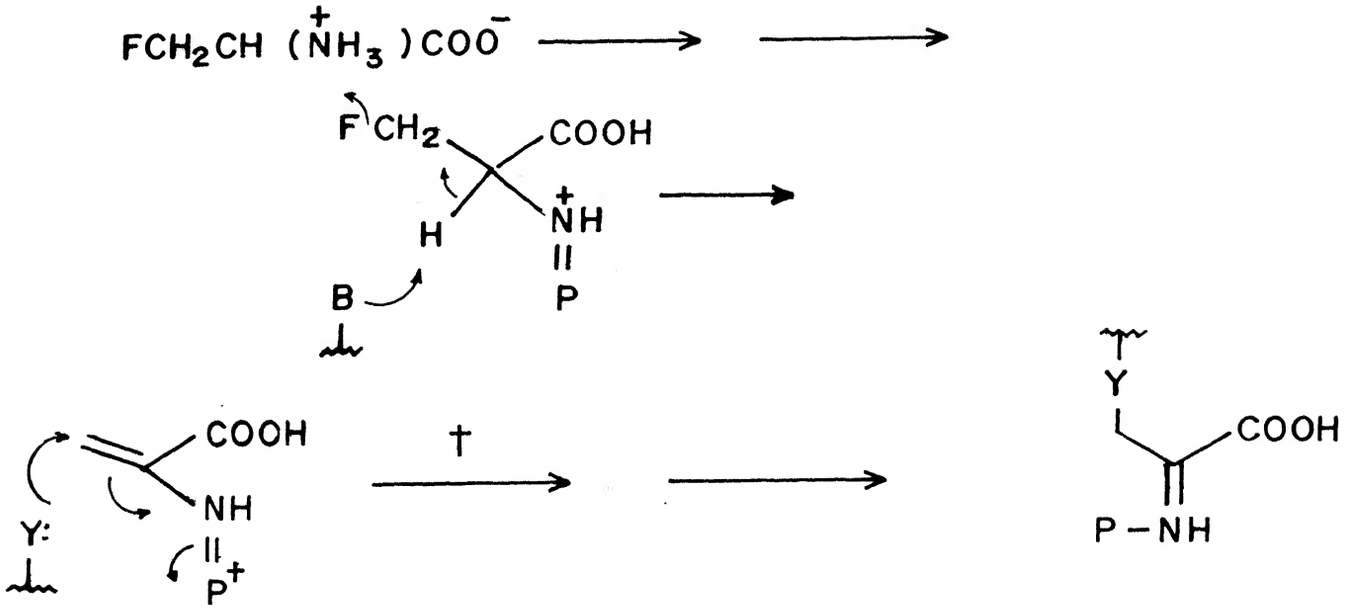
Alanine Racemase (L- Ala ⟶ D Ala)

a. Normal



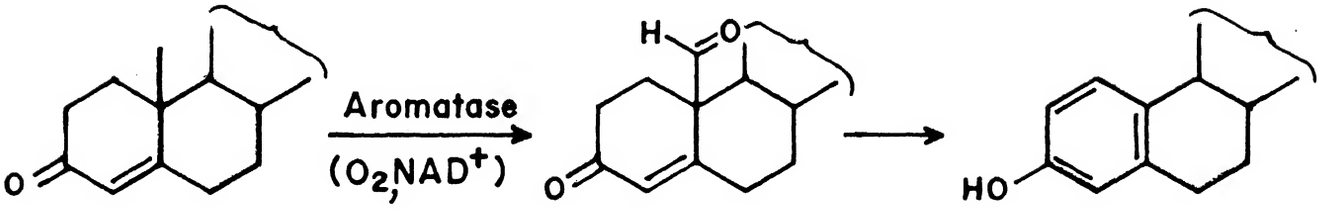
P = Pyridoxal Co-factor

b. Inactivation by β – fluoro alanine



† Suicide inactivation

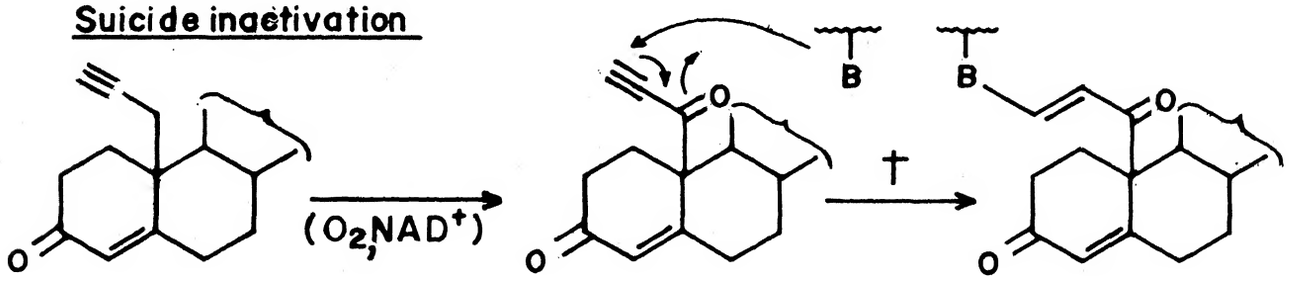
Androgen → estrogen inhibition for cancer therapy



Androgen

Estrogen

Suicide inactivation

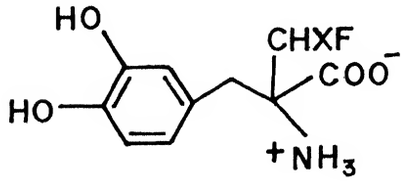


The inhibition of α-amino acid decarboxylases is very much desired in the prevention of a variety of diseases. Some of these are presented in Table 3.2.

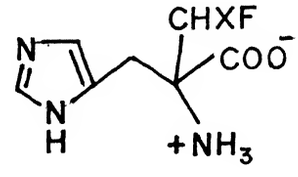
Table3. 2

Enzyme whose inhibition is desirable	*Medicinal benefits*
Aromatic amino acid decarboxylase	Prevent peripheral DOPA decarboxylation and thus enhance drug potency
Histidine decarboxylase	decrease allergenic histamine levels
Ornithine decarboxylase	combat parasitic infections

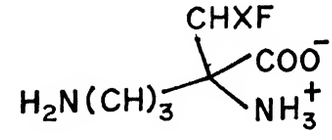
Analogs with fluorine located at β– positions are uniformly effective in inhibiting decarboxylation. The enzymes normally promote the decarboxylation of the pyridoxal Schiff's bases of amino acids. The presence of β -F brings about, instead, a fluoro decarboxylation. The enzyme then invariably adds to the electrophilic intermediates formed (similar to β-fluoro alanine inhibition).



DOPA

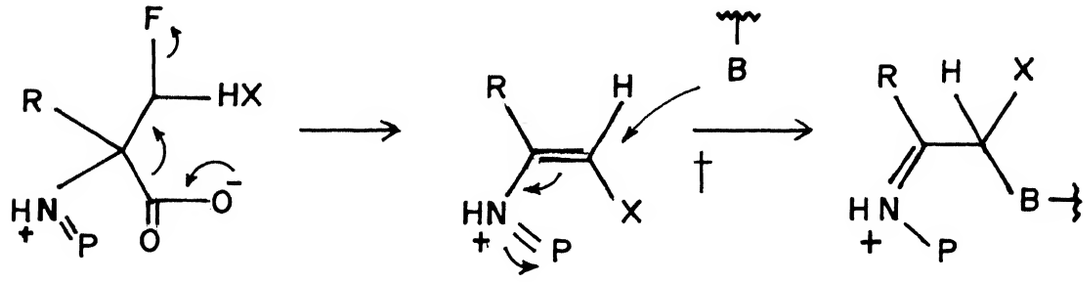


HISTIDINE



ORNITHINE

X = F/H



P = Pyridoxal Co - factor X = H/F

Mechanism based enzyme inactivation by clever structuring of the inhibitors to kinetically trap the enzyme is a very fertile area for drug design and holds much promise.

Leading References

1. Protein structure based drug design. P.J. Whittle and T.L. Blundell. Annu. Rev. Biophys. Biomol. Struct., **23,** 349 (1994).

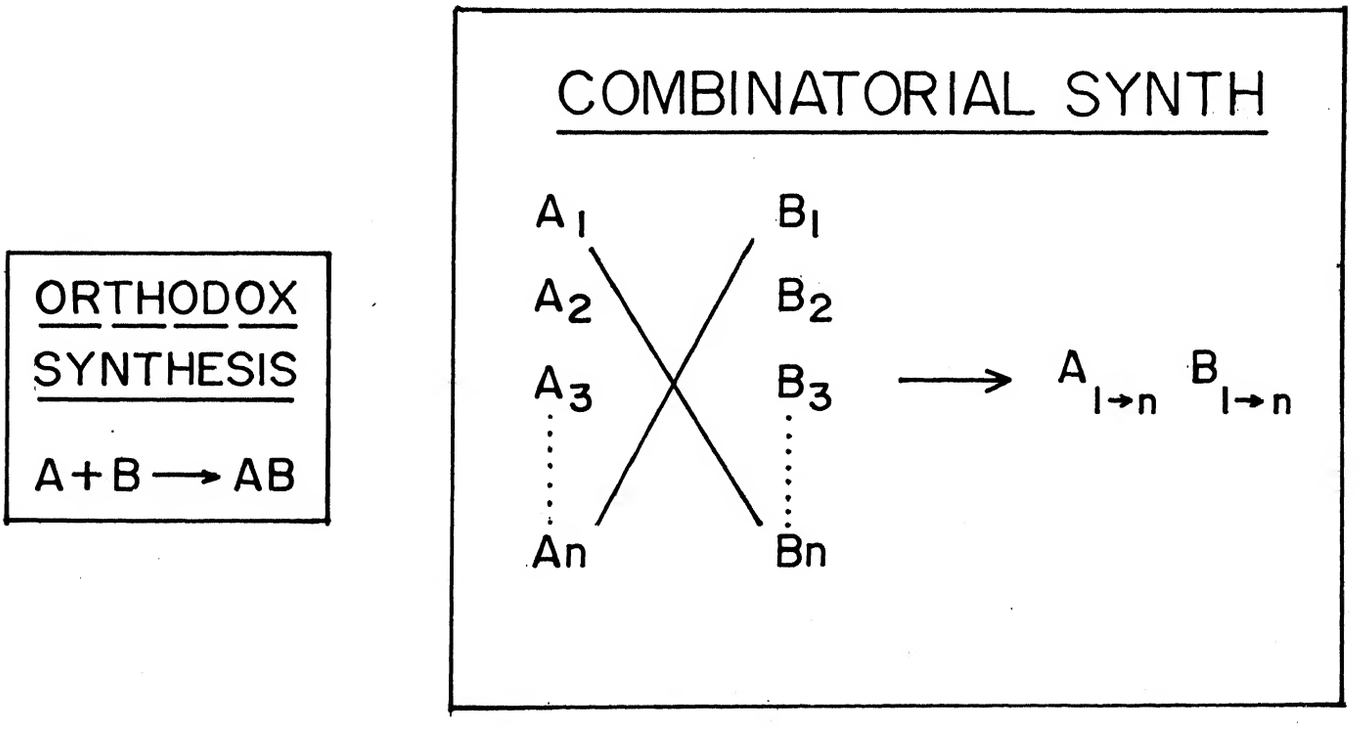
2. Mechanism-based enzyme inactivators in rational drug discovery: Curing diseases with biochemical trojan horse reagents. A. Nangia and P.S. Chandrakala. Curr. Sci., **68,** 699 (1995).

4: COMBINATORIAL SYNTHESIS AND DRUG LEADS

Seldom in the recent past has the imagination of organic chemists has brought forth a new principle, which at once found acceptance and applications in drug discovery, as the domain called combinatorial synthesis. Practicing organic chemists have severely frowned on reactions that gives mixtures. Indeed for this very reason, many leads have been abandoned. In a sense, the chemistry here analyses all possibilities arising out of a reaction that gives mixtures and screens major and minor compounds using the biological activity norms. In the past few years the area has exploded. The purpose of the chapter is to present salient features of this area.

The identification of biological target, the discovery of leads and lead optimization are three important facets of drug discovery. Combinatorial synthesis interphases with the last two protocols. The orthodox and combinatorial synthesis are compared in Chart 4.1.

Chart 4.1



The impact of combinatorial synthesis in the drug scenario can be illustrated with two simple examples.

The reaction of an acid chloride with an amine or alcohol gives, respectively, an amide or an ester. In a combinatorial translation, this single reaction can give 2 x 40 x 40, amides/esters when played with 40 acid chlorides [A series] and 40 amines/alcohols (a series). This can easily be seen from Chart 4.2.

Chart 4.2

$RCOCl\ [A_1 \cdots A_{40}] + R^1X\ [a_1 \cdots a_{40}]\ X = NH_2 / OH$

SET : 1 A SERIES

$A_1 + [a_1 \rightarrow a_{40}] \longrightarrow A_1a_1 + A_1a_2 \cdots\cdots A_1a_n$ 40

$A_2 \longrightarrow \longrightarrow \longrightarrow A_2a_1 \cdots\cdots A_2a_n$ 40

$A_n \longrightarrow A_na_1 \qquad A_na_n$ 1600

SET : 2 a SERIES

$a_1 + [A_1 \rightarrow A_{40}] \longrightarrow A_1a_1 \cdots\cdots A_na_1$

$a_2 \longrightarrow \longrightarrow A_1a_2 \cdots\cdots A_na_2$

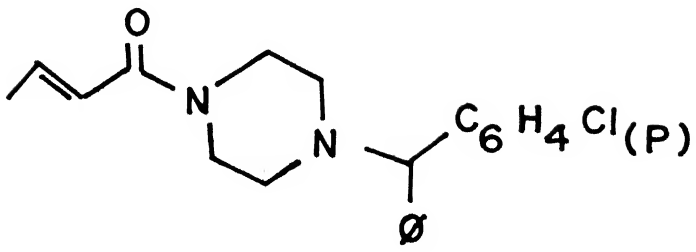
$a_n + [A_1 \rightarrow A_{40}] \longrightarrow a_nA_1 \cdots\cdots A_na_n$ 1600

The simple protocol here thus generates,

i. 2 x 40 sets of 40 amides in each set
ii.. 2 x 40 sets of 40 esters in each set

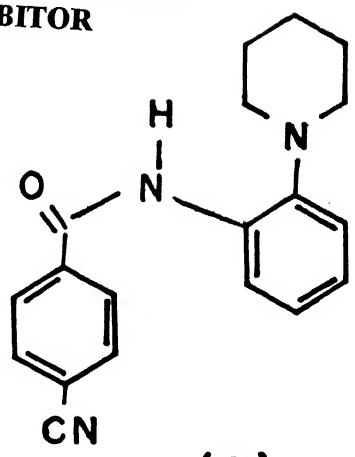
In each set, one component is invariable. Screening the sets afforded two drug leads (1), (2).

NEUROKININ RECEPTOR INHIBITOR



(1)

MATRIX METALLO PROTEINASE INHIBITOR



(2)

One can imagine the impact of this quick drug lead discovery in the industry!

In another illustration, nine alcohols (R_1-OH → R_9-OH) were reacted with 6 isocyanates (a_1 NCO → a_6NCO), to generate 18 sets of urethanes, each having six numbers, where either R or a is constant [chart 4.3].

Chart 4.3

$$R_{1-9}OH + a_{1-6}NCO \longrightarrow R_N O\overset{O}{\overset{\|}{C}}NH a_n$$

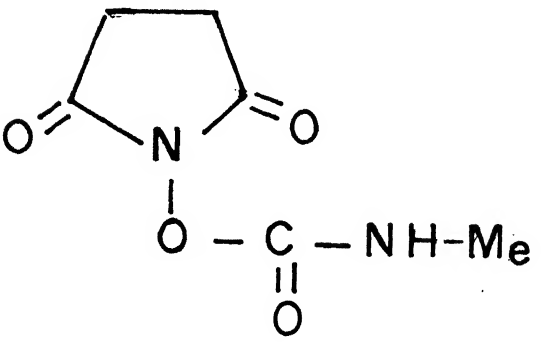
$$R_1 OH + a_1 NCO \longrightarrow R_1 OCONHa_1$$

$$R_N OH + a_n NCO \longrightarrow R_N OCONHa_n \qquad 54$$

$$a_1 NCO + R_1 OH \longrightarrow a_1 NHCOOR_1$$

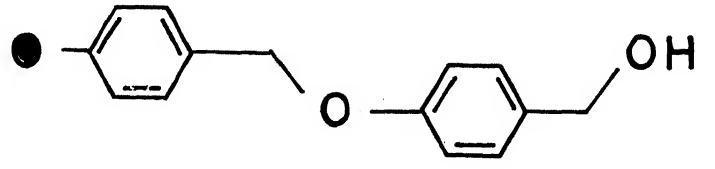
$$a_n NCO + R_N OH \longrightarrow a_n NHCOOR_N \qquad 54$$

Screening of these lead to the acetylcholinesterase inhibitor (3).

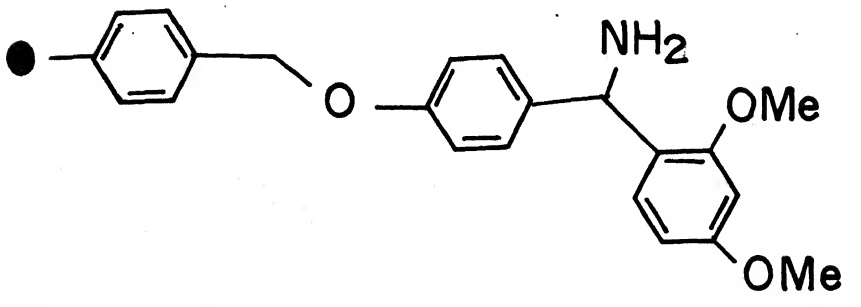


(3)

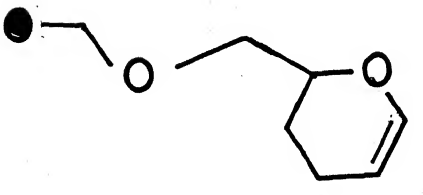
For operational reasons, combinatorial synthesis is best done on polymer supports and three well used supports are presented below:



WANG



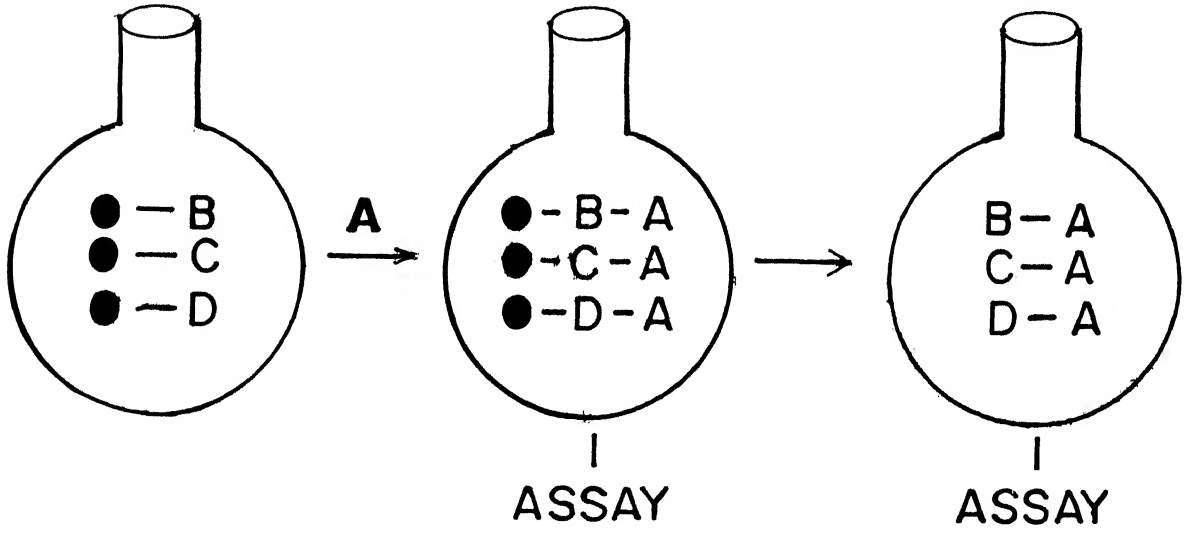
RINK



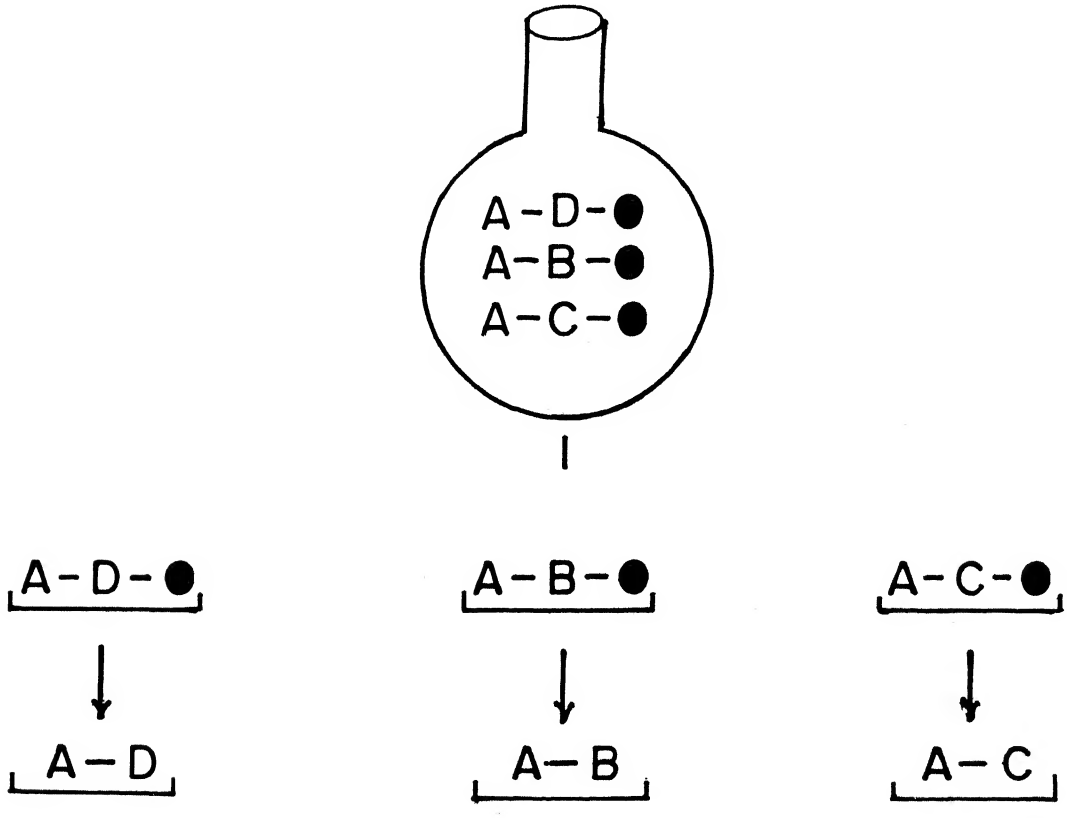
ELLMAN

● POLYMER

As a simple illustration, polymer anchored entities ●--B,●-- C,●-- D can be coupled with A to give, respectively, ●--B-A, ●--C-A and ●--D-A. They could be screened either as bound or free.



Procedures are available for the mechanical separation of polymer bound mixtures. When this is feasible, a single step combinatorial approach can give the results of n orthodox methods, as could be seen below (n=3).



One of the popular protocols in combinatorial synthesis is called the "mix and split" approach. This is illustrated in Chart 4.4, with a 3 unit system[P] and peptidation as the reaction.

In the first step, ●--X, ●--Y, ●--Z are mixed, split into 3 lots, which are coupled with, respectively, X, Y, Z. This would lead to 3 lots having 3 dipeptides each. These are mixed, split into 3 lots and coupled again with, respectively, X, Y, Z. As could be seen from Chart 4.4, this would lead to 3 lots having 9 tripeptides each. Thus, in 2 steps 27 tripeptides are made! In this set up, each operation will triple the number of products and each bead will carry a single sequence.

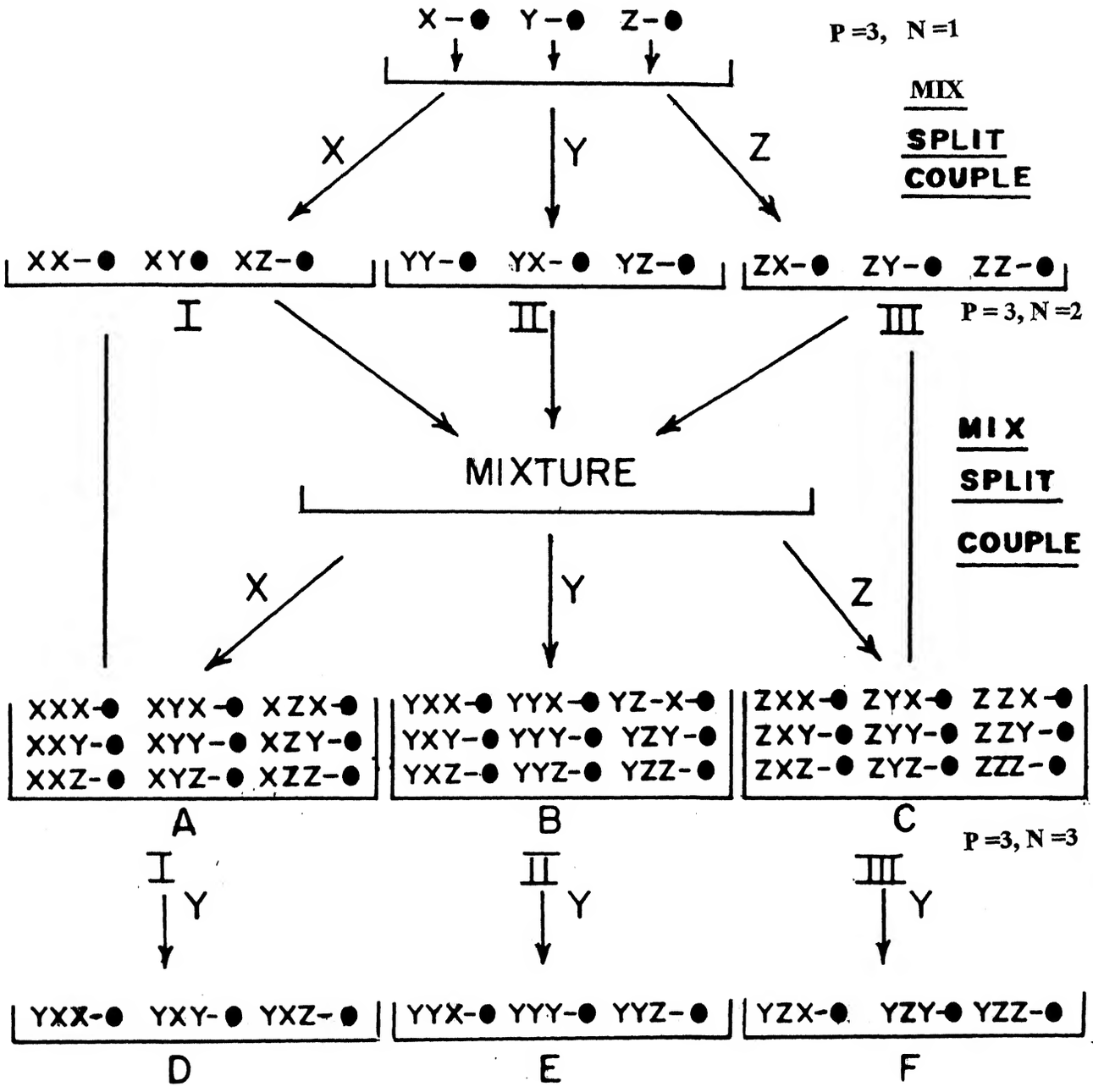
Chart 4.4 shows 3 libraries having 9 compounds each. These could be separately detached from the support to give 27 tripeptides (3 x 9). Let us say that on screening activity was found in flask B. Here all will have Y in the N terminus.

The identification of the active tripeptide can be illustrated by assuming it as YXY, that is, it is present in the Y stream [Chart 4.4]. Now Y can be attached to I, II and III. The active compound will now be 1 in 3, in this case in D. Separation and screening will identify the active compound [Chart 4.4].

This method has been used to construct 34 x 10^6 N-Ac protected hexapeptides and 2.5 x 10^6 pentapeptides that incorporated 19 of the 20 coded amino acid complement. Screening of these has led to strong binders and antigens.

The problems related to screening of such large numbers are formidable and a number of innovative methods have been devised.

Chart 4.4


X-● Y-● Z-●
P =3, N =1
MIX
SPLIT
COUPLE
X
Y
Z
XX-● XY● XZ-●
YY-● YX-● YZ-●
ZX-● ZY-● ZZ-●
I
II
III
P = 3, N =2
MIXTURE
MIX
SPLIT
COUPLE
X
Y
Z
XXX-● XYX-● XZX-●
XXY-● XYY-● XZY-●
XXZ-● XYZ-● XZZ-●
YXX-● YYX-● YZ-X-●
YXY-● YYY-● YZY-●
YXZ-● YYZ-● YZZ-●
ZXX-● ZYX-● ZZX-●
ZXY-● ZYY-● ZZY-●
ZXZ-● ZYZ-● ZZZ-●
A
B
C
P =3, N =3
I
II
III
Y
Y
Y
YXX-● YXY-● YXZ-●
YYX-● YYY-● YYZ-●
YZX-● YZY-● YZZ-●
D
E
F

The μ opiod receptor met-enkephalin can be expected to be one present in a large "small peptide" library. the experimental demonstration of this by "iterative deconvolution" or SURF (synthetic unrandomization of randomized fragments) is illustrative. The hexapeptide library used was, N-Terminal free, amides. The protocol is shown in Chart 4.5 , where O represents a known amino acid and X an unknown.

Chart 4.5

12,84000 hexapeptide library to be screened

↓

321 sublibraries having 400 numbers

↓

Screen O_1 O_2XXXXNH$_2$ for most active O_1 , O_2

↓

O_1O_2 identified as Tyr-Gly or Trp-Trp

↓

Tyr-Gly-XXXXNH$_2$ was deconvoluted by identification of X_3 ,

↓

with 1 each of the 20 code complement

↓

Of the 20 libraries of the type Tyr-Glu-OXXXNH$_2$,

↓

Tyr-Gly-Gly was most active

↓

Tyr-Gly-Gly XXXNH$_2$ was deconvoluted by identification of X_4

↓

with 1 each of the 20 code complement

↓

Of the 20 libraries of the type Tyr-Gly-Gly-OXXNH$_2$,

↓

Tyr-Gly-Gly-Phe was most active

↓

Tyr -Gly-Gly- Phe-XXNH$_2$ was deconvoluted by identification of X_5

↓

with 1 each of the 20 code complement

↓

Of the 20 libraries of the type Tyr-Gly-Gly-Phe-OXNH$_2$,

↓

Tyr-Gly-Gly-Phe-Met was most active

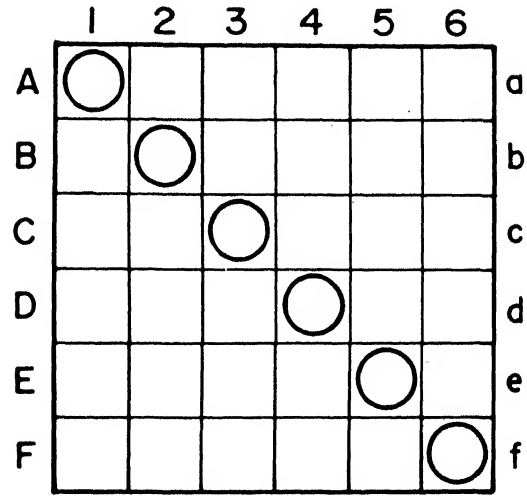
↓

Tr-Gly-Gly-Phe-Met IS THE NATURAL ENKAPHELIN!

The "extra" sixth best was identified as Ala by the above protocols.

Another useful method to find the "highest affinity" peptide is by "scanning" six positional combinatorial libraries [A-F], such that each contained all possible combinations of 18 of the 20 code complement (except Trp and Cys). In the general set $X_1X_2X_3X_4X_5X_6NH_2$, 1-6 locations were tagged with known amino acid "O" to provide representation shown in chart 4.6.

Chart 4.6

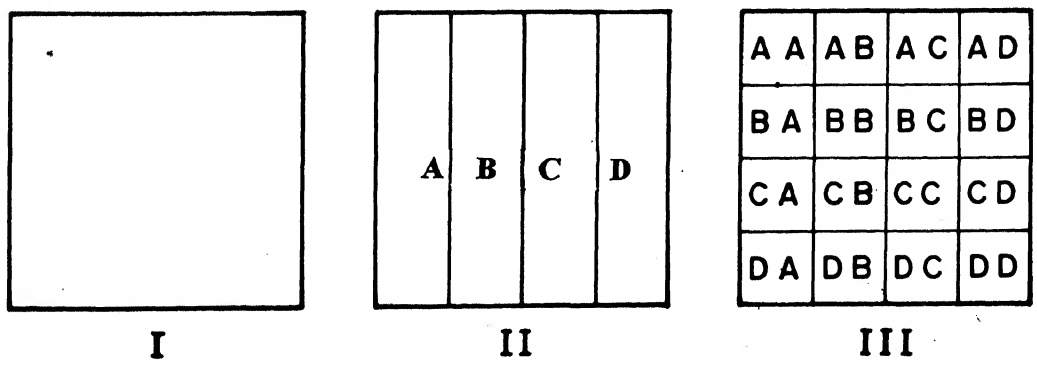


This led to formulation of the highest affinity peptide as abcdef [$YGGFMYNH_2$].

By merging of techniques of solid state chemistry and photolithiography an array of peptides can be displayed which are spatially addressable.

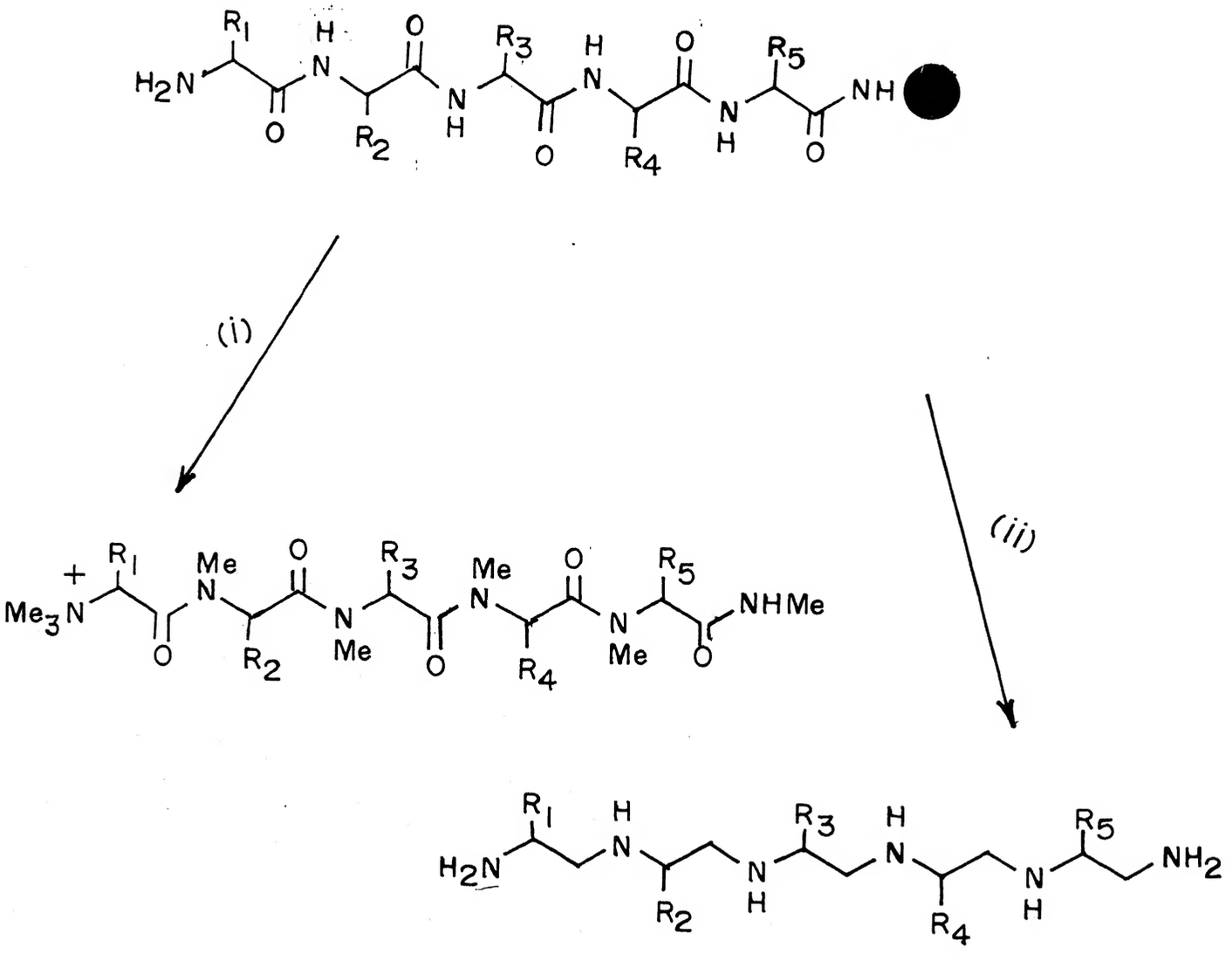
The square plate (I) is functionalized with amino groups, which are protected with the photolabile nitro veratryloxycarbonyl (NVOC) group [Chart 4.7].

Chart 4.7



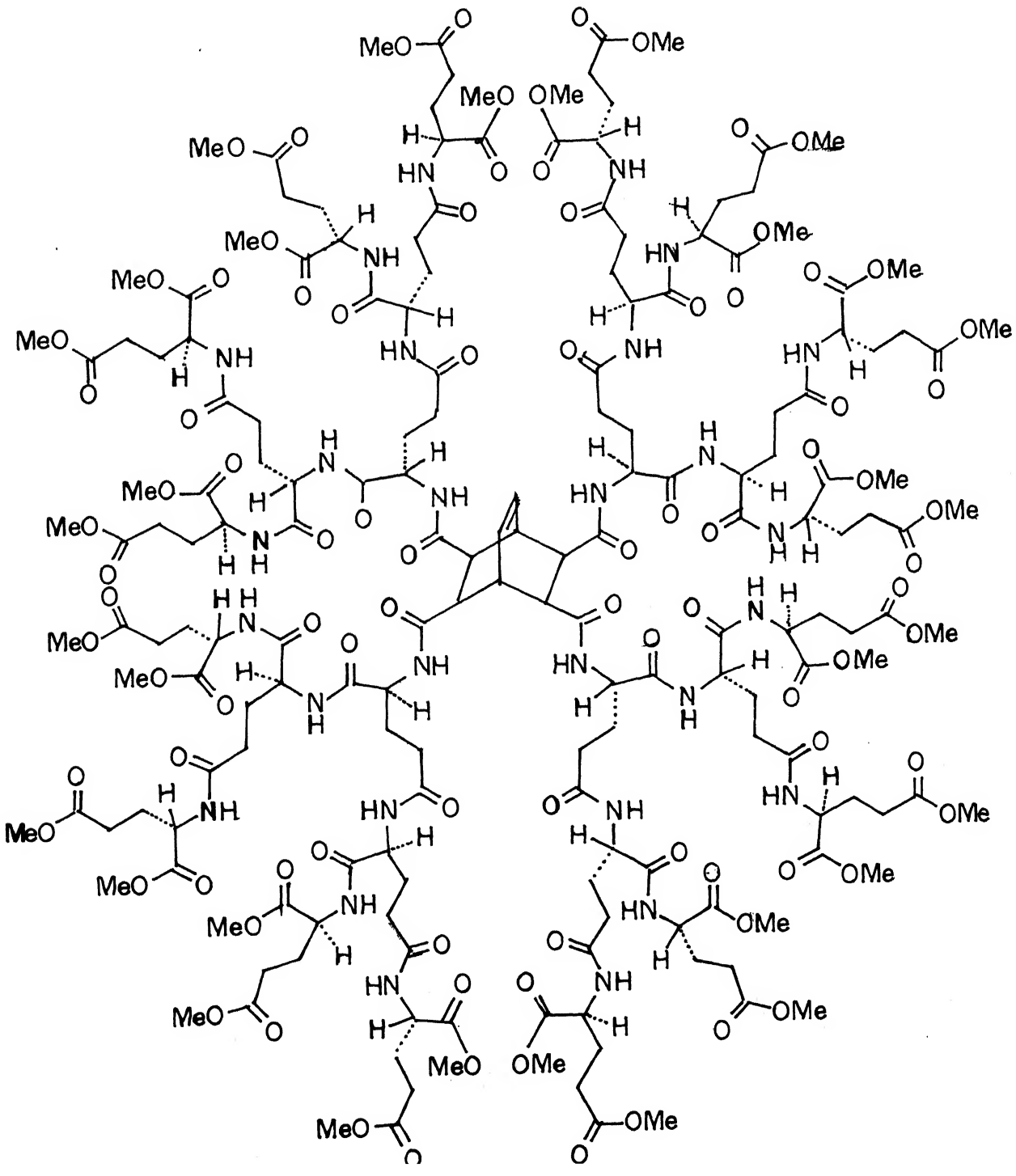
By masking I, vertical strips (A, B, C, D - II) are sequentially illuminated to remove NVOC and expose the NH_2 groups, which are coupled to A-NVOC, B-NVOC, C-NVOC and D-NVOC in stripes, respectively, A, B, C and D (II). Orthogonal stripes are then generated by masking and illumination and coupled to NVOC protected A, B, C, D, to give III [Chart 4.7]. The protocols could be repeated to grow peptide chains of specific sequence and specific location.

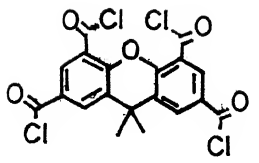
As shown in below, daughter libraries can easily be generated by normal synthetic protocols.



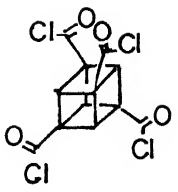
A core generated dendrimer is shown in Chart 4.8 from bicyclo (2.2.2) octene. A similar protocol can be used to generate disk-like and sphere-like combinatorial libraries from (4) and (5) respectively.

Chart 4.8





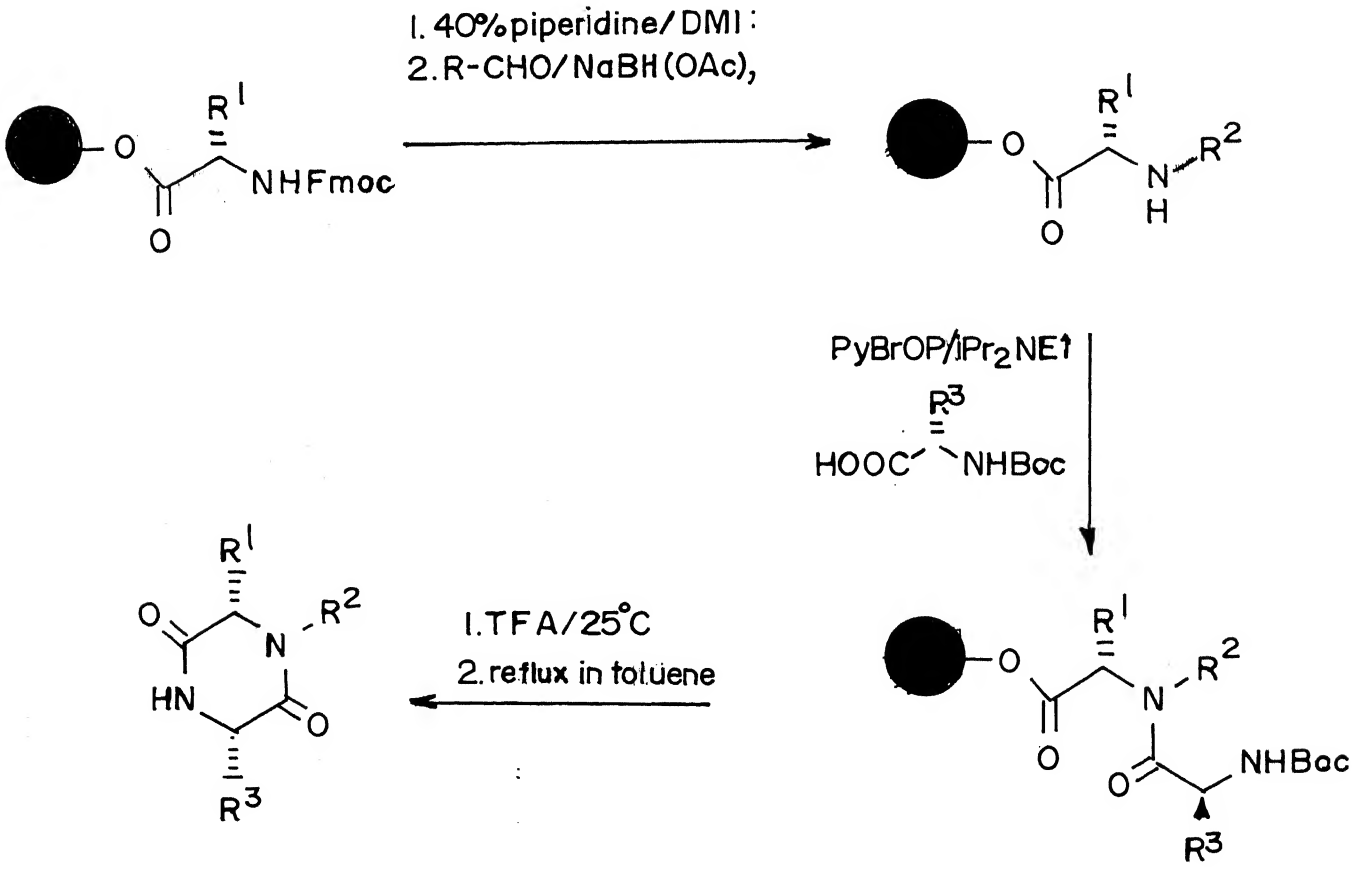
(4)



(5)

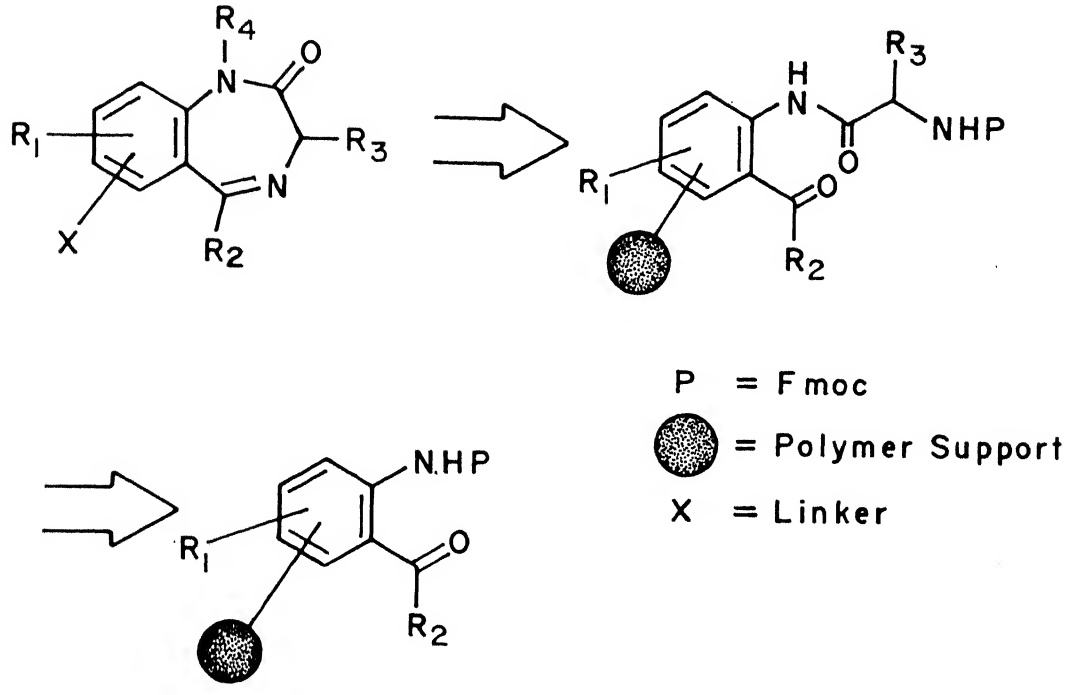
Diketopiperazines [DKP] are of current interest as catalysts in asymmetric synthesis. A 1000 DKP library has been constructed as shown in Chart 4.9. Three of the 4 sites of DKP has been taken advantage of to generate molecular diversity.

Chart 4.9



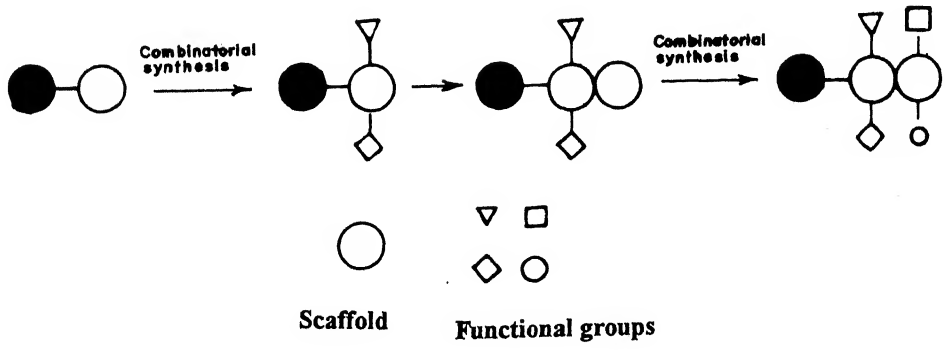
Of interest is the generation of a "diazepam" library, having 4 sites [R_1, R_2, R_3, R_4] to secure diversity as shown, in a "retro synthetic mode", in Chart 4.10.

Chart 4.10



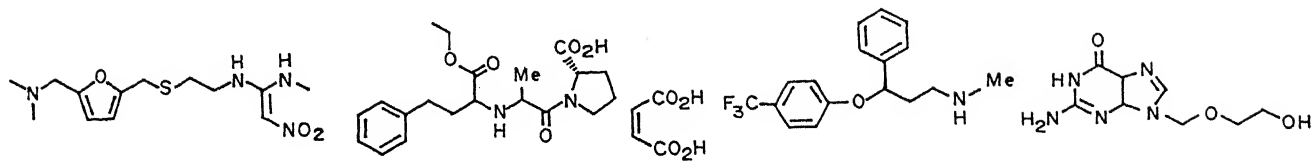
The construction of a biphenyl library is interesting. Here, polymer bound phenyl is split, functionalized by various ligands, mixed, coupled with a phenyl group, split, functionalized with various groups and mixed to afford biphenyls with an unbelievable diversity in substitution [Chart 4.11].

Chart 4.11



In Chart 4.12 is presented the best 20 selling drugs in 1994.

Chart 4.12

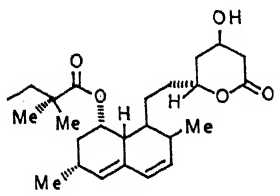


1. Zantac (4·0)

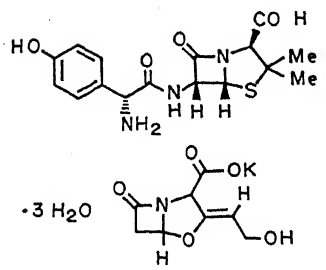
2. Vasotec (2·0)

3. Prozac (1·6)

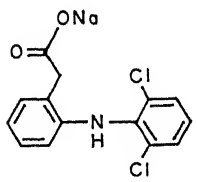
4. Zovirax (1·5)



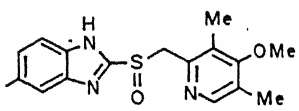
5. Zocor (1·4)



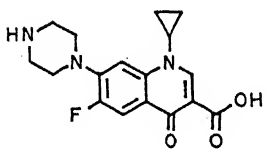
6. Augmentin (1·3)



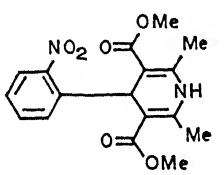
7. Voltaren (1·3)



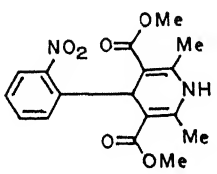
8. Losec (1·3)



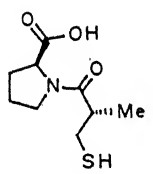
9. Ciproxin (1·3)



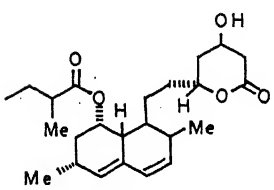
10. Procardia (1·2)



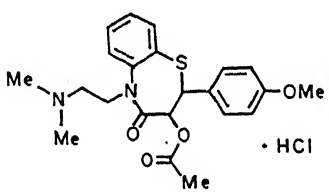
11. Adalat (1·2)



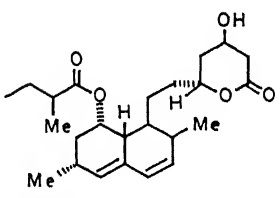
12. Capoten (1·2)



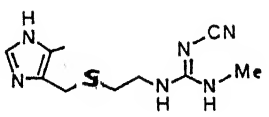
13. Mevacor (1·1)



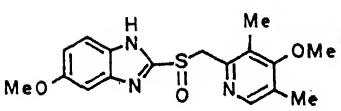
14. Cardizem (1·1)



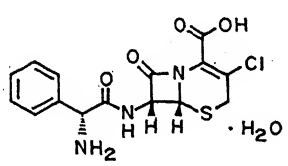
15. Mevalotin (1·1)



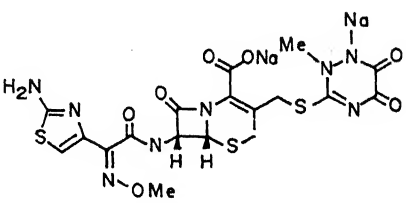
16. Tagamet (1·0)



17. Prilosec (0·9)



18. Ceclor (0·9)



19. Rocephin (0·9)

Mixture of estrogens

20. Premaxin (0·8)

Top 20 ethical pharamceuticals prescribed in 1994. [numbers in parenthesis represent sales in billions, US$]

This list, when compared to the year 1992 [chapter 1, Table 1]. see deletions. Indeed all of the top 20 drugs, just 2 years ago are not here. The fact is, newer and better drugs are being discovered continuously. Thus, search for new drugs will go on. As a rule, the second generation drugs are similar to those previously in vogue. In this context combinatorial synthesis offer great advantages. For example, if combinatorial libraries can be created from drugs in Chart 4.12, the chances of discovery of better drugs would be high. True, at present unlike biopolymers, preparation of combinatorial libraries of small molecules pose greater challenges, which, as has been illustrated in the present chapter with few examples, will surely be overcome.

The above account on combinatorial synthesis largely dwelt with design and methodologies rather than details. Every aspect of this science is undergoing revolutionary changes and in the process an explosion in information. The basic approaches are transformed to specialized ones of increasing complexity where visible human ingenuity can be discerned. Automation is taking over many of the iterative protocols. A welcome symbiosis of methodologies in organic synthesis and protocols in biology spell a great future to the nascent domain of combinatorial synthesis.

Leading References

1. Combinatorial synthesis: The design of compound libraries and their application to drug discovery. N.K. Terett, M. Gardner, D.W. Gordon, R.J. Kobylecki and J. Steele. Tetrahedron, **51**, 8135 (1995) (Report No. 377).

2. Combinatorial chemistry. Lowe, G. Chem. Soc. Rev., **24**, 309 (1995).

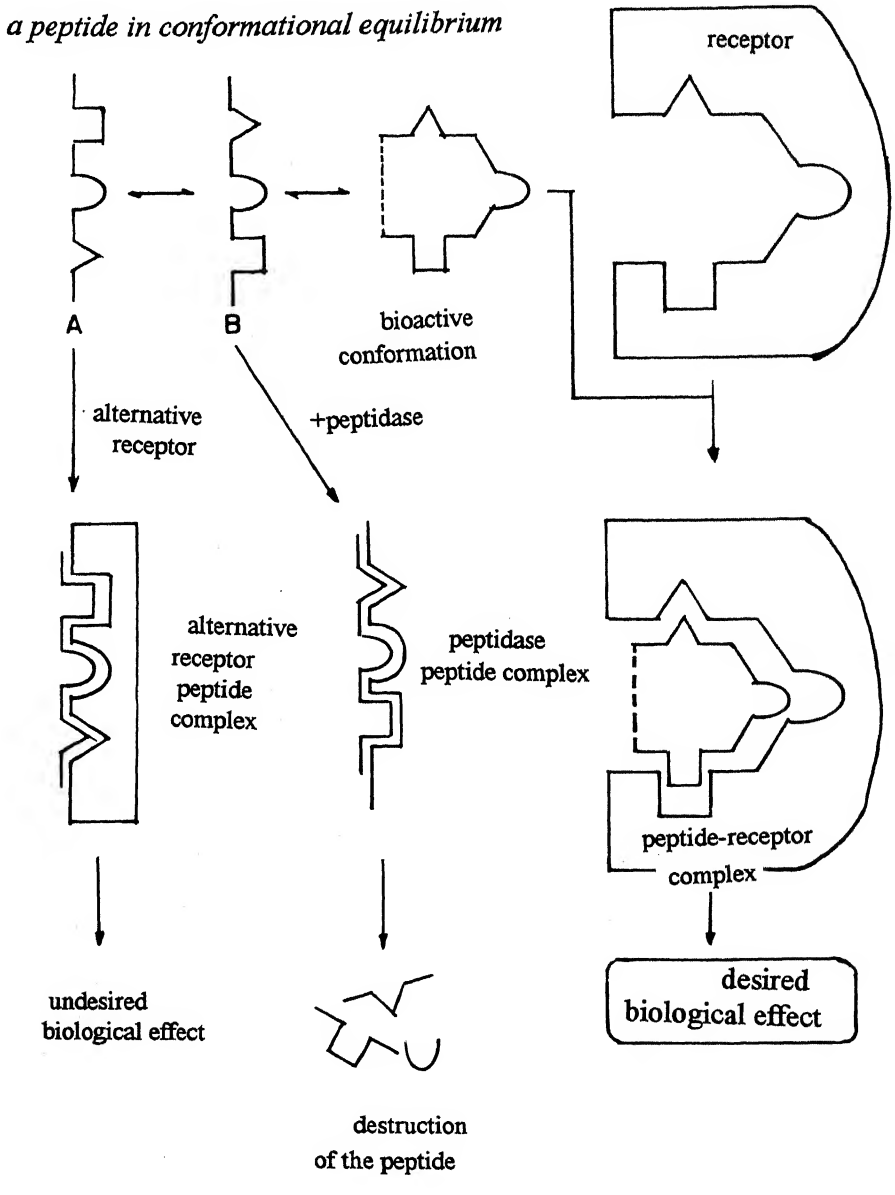
3. "Combinatorial chemistry" Special issue. Acc. Chem. Res., **29**, No. 3, (1996).

4. "Combinatorial chemistry" Special Report, C&EN, February 12, 1996.

5: Peptidomimetics

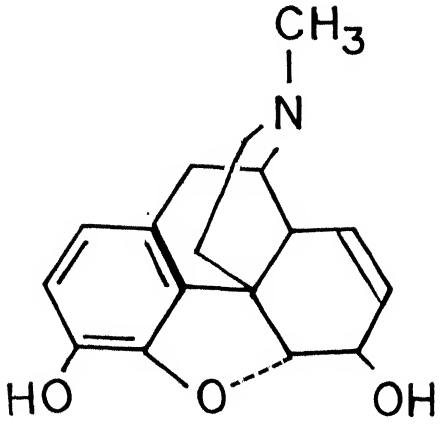
This chapter will be an overview of problems and prospects of generating non-peptide leads, often guided by a peptide model. A peptide, however active it may be, has a slim chance of becoming a drug. The amount of information available on the role of peptides as regulators of biological events has grown exponentially. Yet they have found little use as pharmaceutics [Table 1, Chapter 1; Chart 4.12]. Typically, small peptides, when intravenously administered, have a half-life ranging from seconds to minutes. They are seldom active when orally given.

The conformational flexibility of peptides create several problems. Generally, of the many possible conformations, only one would be active in the formation of the receptor complex and the resulting biological effect. Others can trigger harmful effects or could be degraded by protease sites recognition.



Thus, what needs to be done is either to freeze the desired conformation or design non-peptides which will bind to the receptor site readily. Analysis of these two issues forms the focus of this presentation. Such a discussion should start with morphine (1).

The opioids are classical examples of non-peptide ligands that were later discovered to be mimetics of endogenous peptides. Morphine (1) imitates the effect of the 31 residue, endogenously produced β-endorphin (2). This example demonstrates unequivocally that it is possible to use a small compound as a substitute for a relatively large peptide .



(1)

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
Gln-Thr-Pro-Leu-Val-Thr-Phe-Lys-Asn-Ala-
Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-

(2)

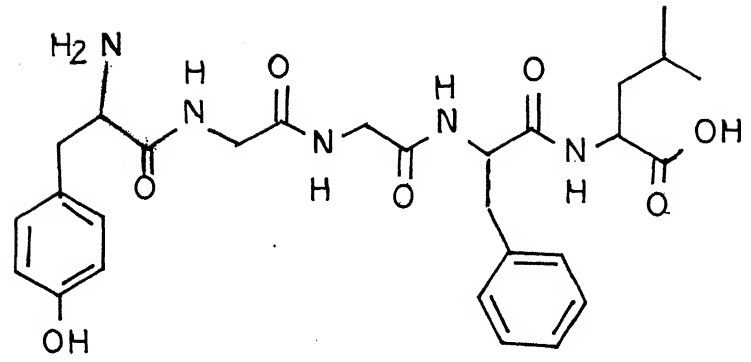
Developments in biology, relating to opioid receptors, have made it possible to create structures that mimic opioid peptides. Three types of opiate receptors (μ, κ, δ) are implicated in opioid action. Dymorphin (3) and enkephelin (4) are endogenous ligands for κ, δ receptors. A comparison of (3) and (4) would show that the N terminals have common Tyr Gly Gly Phe sequence. For drug design, these could be identified as, "message" [Tyr] spacer [Gly-Gly] and "address" [Phe] regions. Naltrindole (5), synthesized on this principle, proved to be the first non-peptide antagonist for the δ opioid receptor.

Another excellent example of crafting peptidomimetics pertains to the pituitary hormone oxytocin (6).

The orally active oxytocin antagonist (7) was designed on the basis of overlap with (6), where the Tyr part corresponds to the aniline part and the isoleucine to camphor!

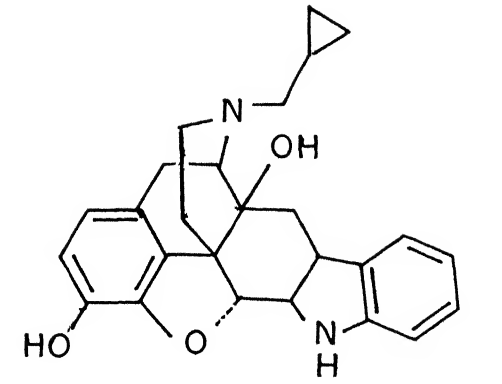
Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys

(3)



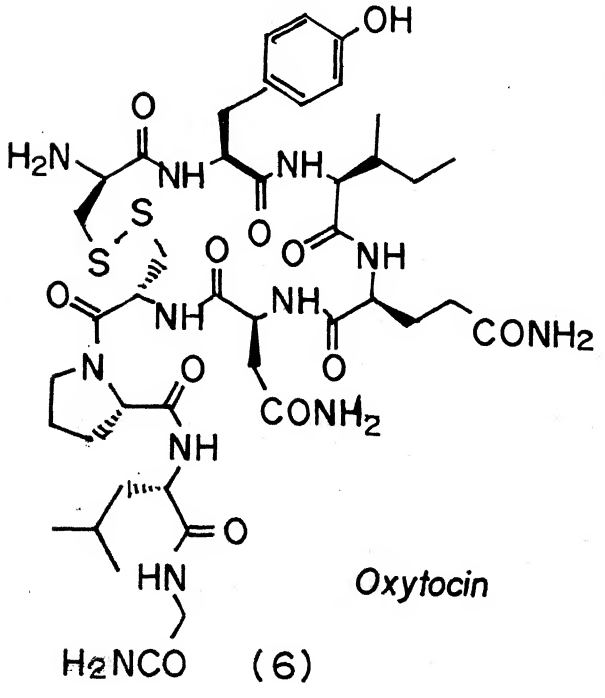
mossage spacer address

(4)



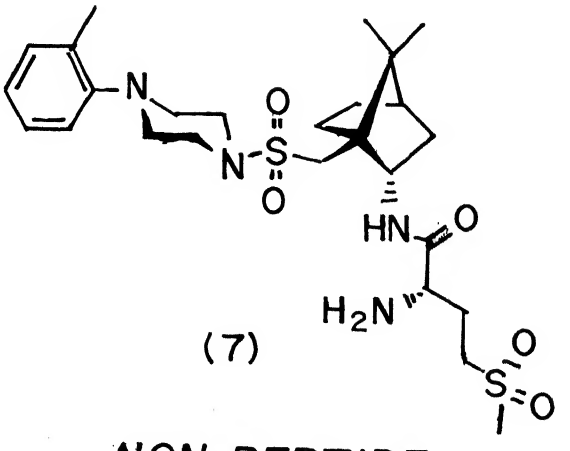
message spacer address

(5)



Oxytocin

(6)



(7)

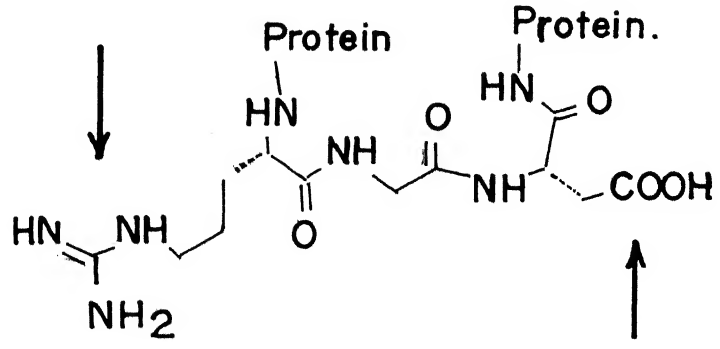
NON-PEPTIDE

L-368,899

pA_2=8.86 (OT; rat uterus)

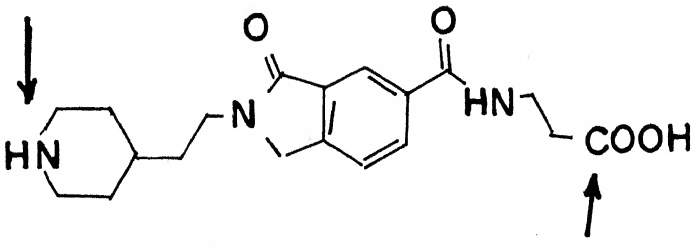
30% orally biavailable in humans

The RGD [Arg-Gly-Asp] motif (**8**) is the minimum sequence required for receptor binding to promote platelet aggregation. Antagonists of RGD are therefore important in cardiovascular therapy. Non-peptide RGD antagonists have been designed (**9-11**). Indeed, (**11**) is orally active.



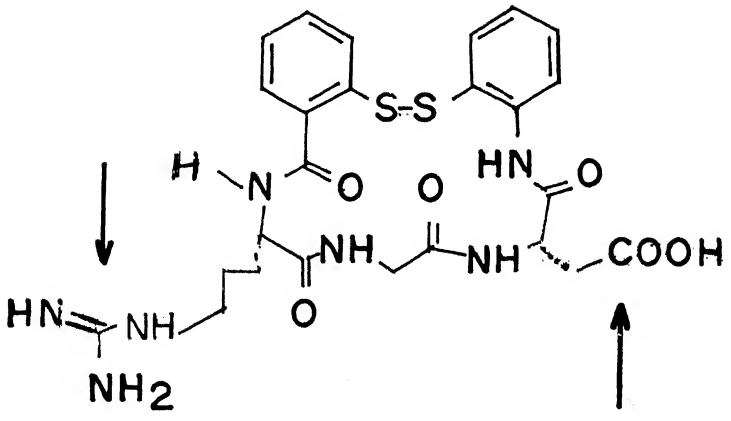
RGD Sequence in fibrinogen α-chain

(8)



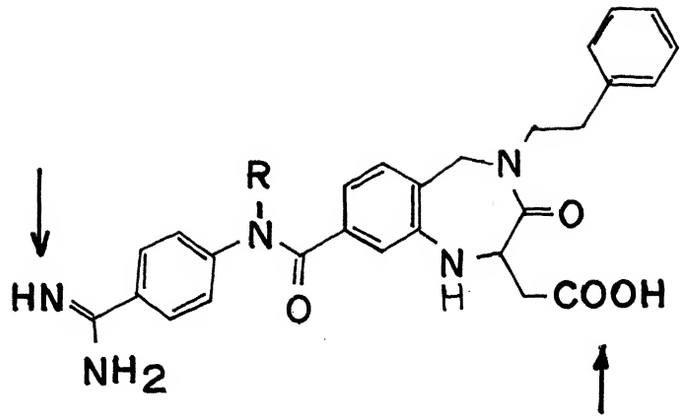
L-709,708 IC50 = 25 nM
(inhibition of platelet aggregation)

(9)



Peptide-based peptidomimetic
Ki = 2 nM

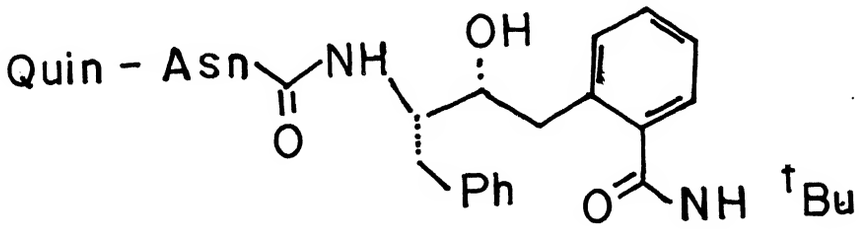
(10)



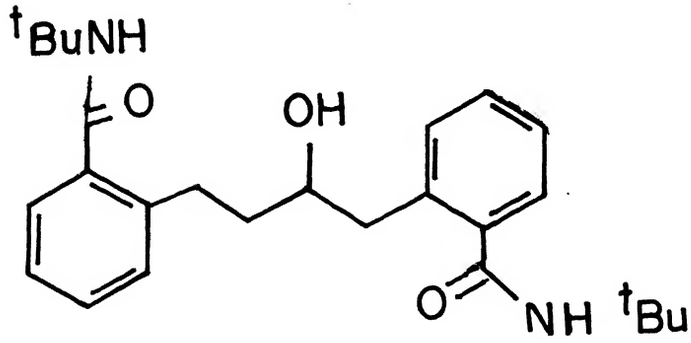
Non peptide peptidomimetic
R = H or Me, Ki = 2nM
IC50= 150nM (inhibition of platelet aggregation)

(11)

The transformation of a lead HIV proteinase inhibitor (**12**) to non peptidic, yet active compounds **13-14**, is illustrated below

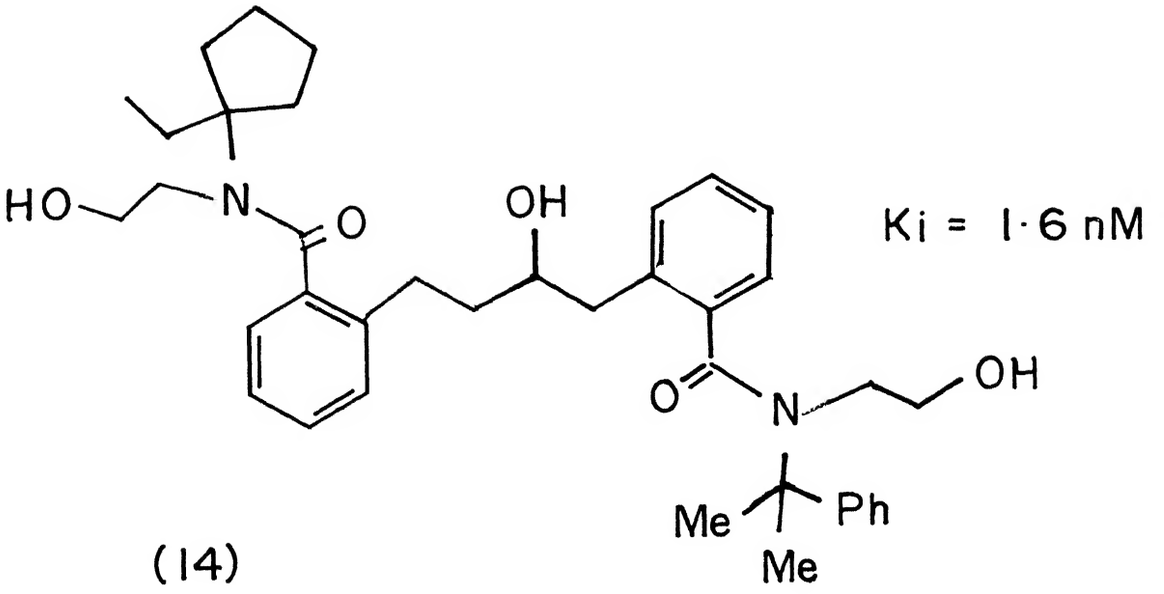


(12) PEPTIDE LEAD



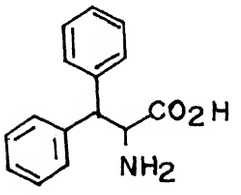
Ki = 18 μM

(13)

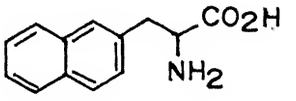


(14)

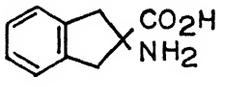
Several avenues exist for the crafting of either conformationally restricted or metabolically stable peptidomimetics at the amino acid level. Alkylation [N_1,C_α), increasing of steric bulk and tethering are some common protocols. Some possibilities with Phe (15)-(21), Leu (22) and Trp (23) are illustrated.



(15)



(16)



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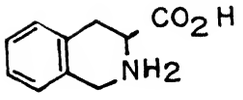

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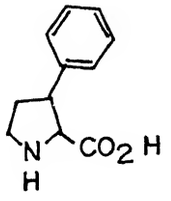
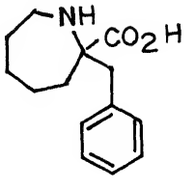


(19)



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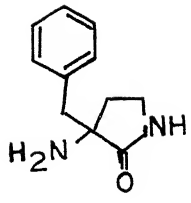

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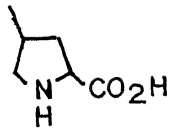

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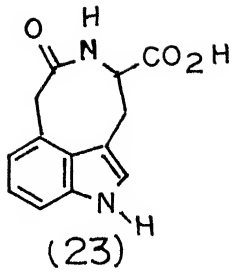
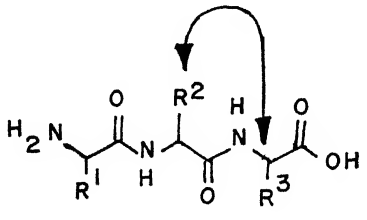
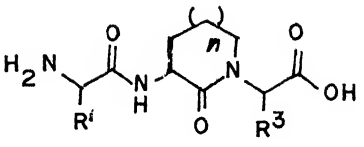


(23)

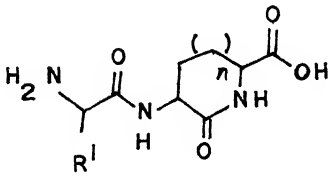
Bridging of two neighbouring amino acids in a peptide leads to a variety of interesting peptidomimetics as could be seen from **24-30**. A surprising number of hormones can be brought into this category. For example, **30** is a very potent ACE inhibitor.



24



25



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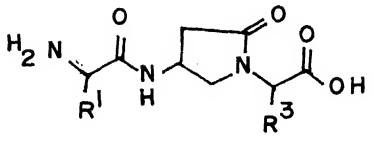

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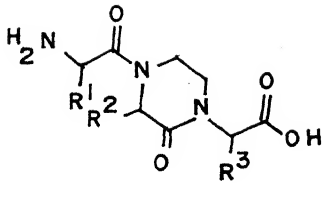

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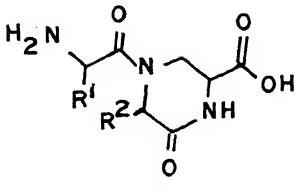

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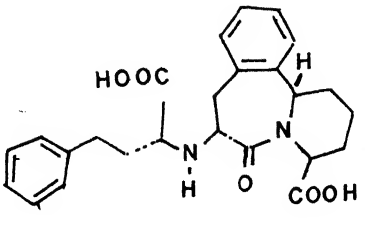
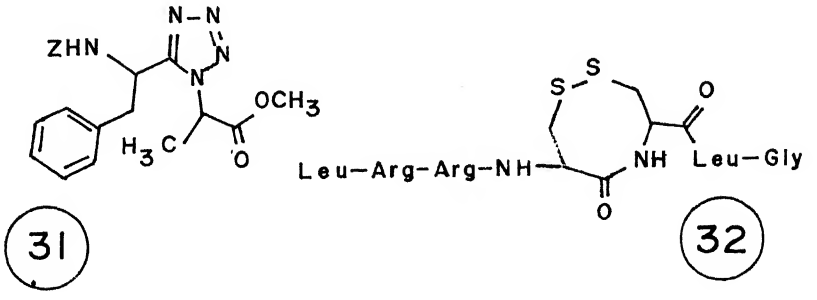
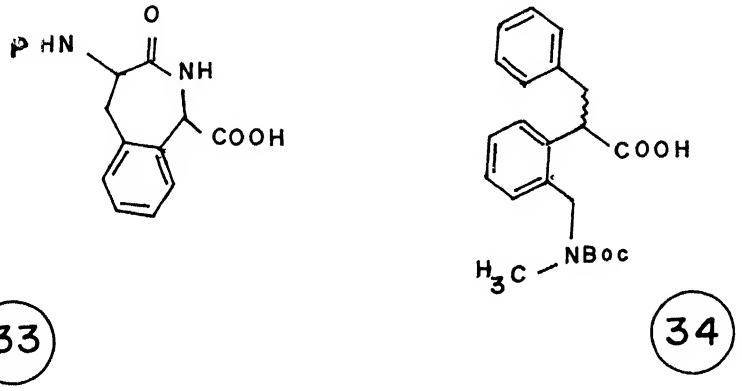


30

The thermodynamically disfavoured cis peptide bonds are in several cases implicated in receptor interaction. In this situation, the available active conformation would be too low. To overcome this, cis peptides or their surrogates can be crafted into the peptide manifold, as exemplified with **31-34**. Indeed the incorporation of **(34)** into a cyclic peptide analog of somatostatin gave a biologically active compound.



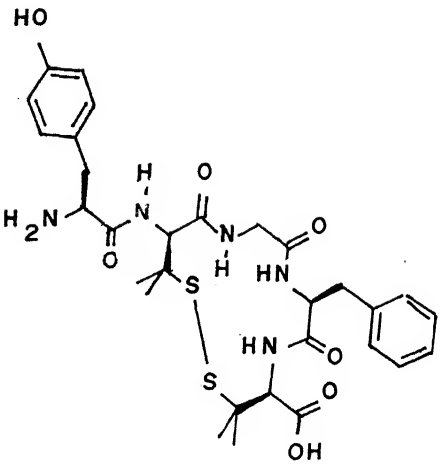
31 32



33 34

Restrictions on the peptide conformation are possible by limiting the flexibility of the peptide strand through cyclization of side chains, which are not involved in receptor recognition.

Compound **(35)**, formed by an 2SH $\rightarrow$ -S-S- change of penicillamine is a cyclic enkephelin analog with selectivity for the δ opiate receptor. Compound **(36)**, arising by amidation of proximate lysines and aspartic acid, and, **(37)** formed by succinoylation of proximate lysines are potent ligands for cholecystokinin receptors.



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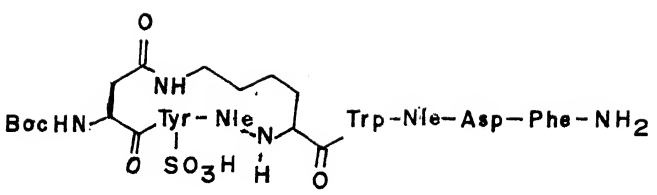

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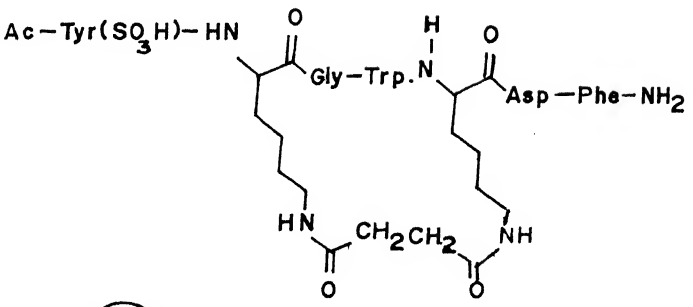
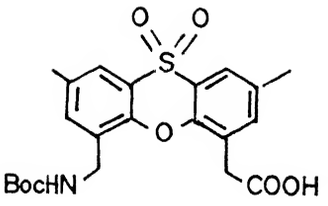
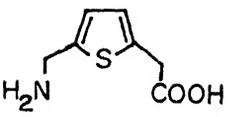
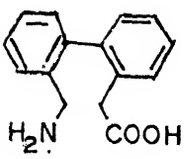


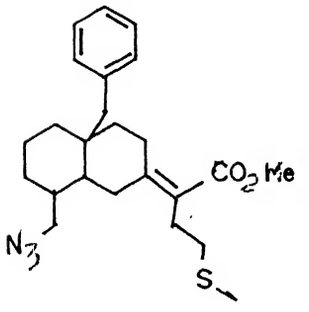
37

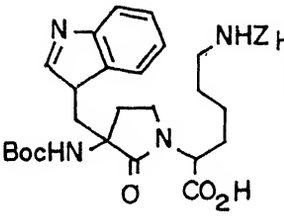
A β-turn is generally a segment of 4 amino acids (i → i + 3) that occurs when a peptide strand changes direction. The β-turn is a structural motif common to many biologically active cyclic peptides and possibly for the biologically active form of linear peptides. For this reason it is the most imitated secondary structural motif. Some interesting β turn mimics are shown below. Most of these are not active after incorporation to model peptides. However striking results have been obtained by this strategy. For example (38) mimics a β-turn in the sequence Tyr-Ser-Gly-Ser-Thr, a part of the monoclonal antibody against influenza virus (39). Compound (38), the first example of a low molecular weight immunoglobulin mimic, is resistant to proteases and imitates the binding and function of the native antibody.

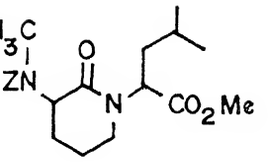

O
O
S
O
BocHN
COOH

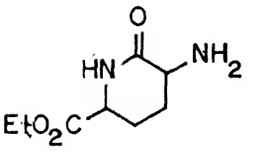

S
H_2N
COOH

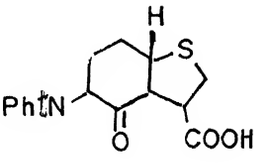

H_2N
COOH

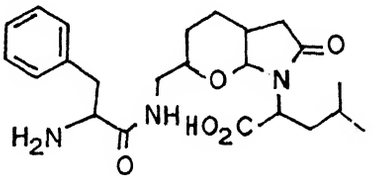

CO_2Me
N_3
S

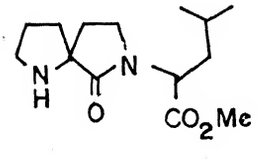

N
NHZ
BocHN
N
O
CO_2H

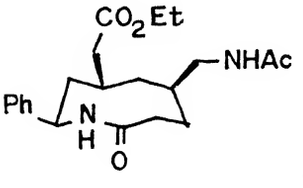

H_3C
ZN
O
N
CO_2Me

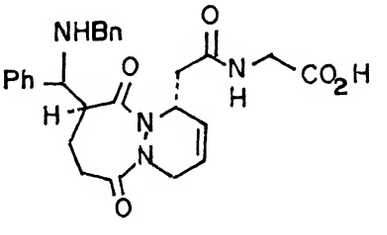

O
HN
NH_2
EtO_2C

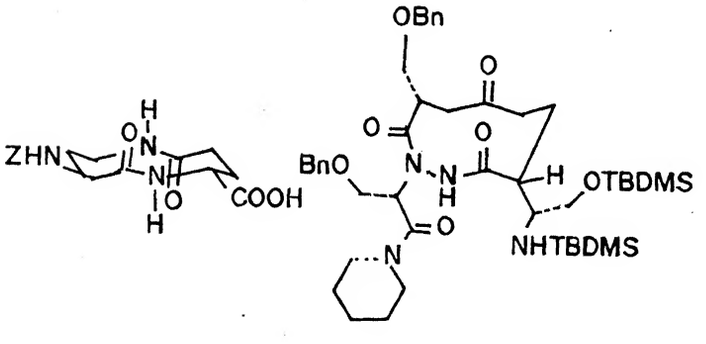

H
S
PhtN
O
COOH

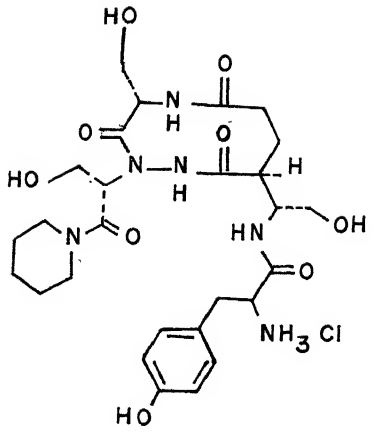

O
O
N
H_2N
NH
HO_2C
O


N
H
N
O
CO_2Me


CO_2Et
NHAc
Ph
N
H
O


NHBn
O
Ph
H
O
N
H
CO_2H
N
N
O


OBn
O
O
ZHN
H
N
O
NH
O
H
COOH
BnO
N
N
H
O
H
OTBDMS
NHTBDMS
N



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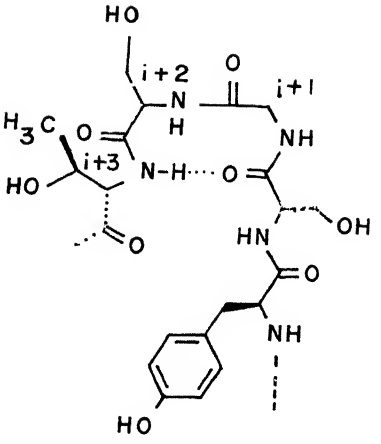

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Modification of the peptide backbone leads generally to an increase in biological half-life and without significantly affecting the conformation. A popular approach to peptide backbone modification is the replacement of the -NHCO- with "equivalent" groups (-$NHCH_2$, CH=CH, $COCH_2$, OCH_2, SCH_2, etc.). In Chapter 1, the use of retro inverso peptides, whose side chains have the same disposition as the parent, as ligands for CD receptors, were discussed. Because chiral inversion at each centre, they cannot be degraded by proteases.

Apart from ingenuity, the design of peptidomimetics is based on structural and biological inputs. The vibrancy of the domain is reflected from the fact that peptidomimetics in numerous domains, such as opioids, tachykinins, somatostatin, gastrin releasing peptide, cholecytokinin, angiotensin II, endothelins and several others, have been designed with the result that the number of peptidomimetics runs to several thousands!

Leading References

1. Peptidomimetics for receptor ligands - Discovery, development and medical perspectives. A. Giannis and T. Kolter. Angew. Chem. Int. Ed. Engl., **32**, 1244 (1993).

2. Peptidomimetics and small molecule drug design: Towards bio availability and *in vivo* stability. N. Beeley. Tibtech, **12**, 213 (1994).

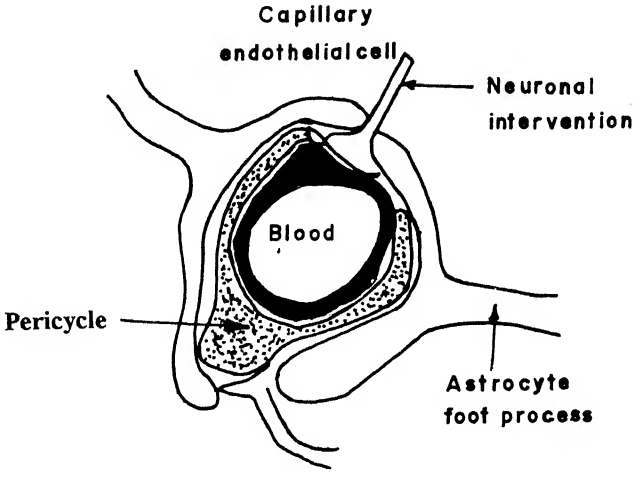
3. Recent advances in the design and synthesis of small molecule mimetic drugs. W.M. Kazimierski. Tibtech, **12**, 216 (1994).

6: STRATEGIES FOR DRUG DELIVERY THROUGH THE "BLOOD-BRAIN BARRIER" [BBB]

The next decade will have greater focus pertaining to the discovery of drugs that could combat dysfunctions in the brain. Human sufferings from this type of ailments are immense. The progress here has been slower than in other fronts. In the past decade enormous progress has been made towards the understanding of processes that take place in the brain. Utilization of these developments will require the parallel development of practical strategies for delivery of drugs *in vivo*, through the endothelial wall of capillaries in the brain, the blood-brain barrier (BBB). The brain and spinal chord constitute the only organ to be perfused by capillaries having such a barrier, which excludes the uptake into the brain of circulating molecules that do not have access to several specialized transport systems within the barrier. The challenge is to develop effective drug delivery strategies to the brain, in parallel with ongoing drug discovery programs.

A cross section of brain blood vessel is shown in Chart 6.1. As could be seen, the vessel is tightly surrounded by endothelial cells, which constitute the BBB. A wall protects these, though occasionally neuron intervention can directly innervate the endothelial cell. Pericytes are contractile and present antigens. The astrocyte foot process constitutes > 98% of the brain surface of the vessel.

Chart 6.1



Circulating molecules can gain access to interstitial fluid in the brain, either by free diffusion or by facilitated transport. Trans-cytosis comprises of three sequential steps:

1. receptor mediated endocytosis at the blood side of BBB,
2. diffusion through the endothelial cytoplasm (distance ~ 300 nm), and
3. exocytosis at the brain side of BBB.

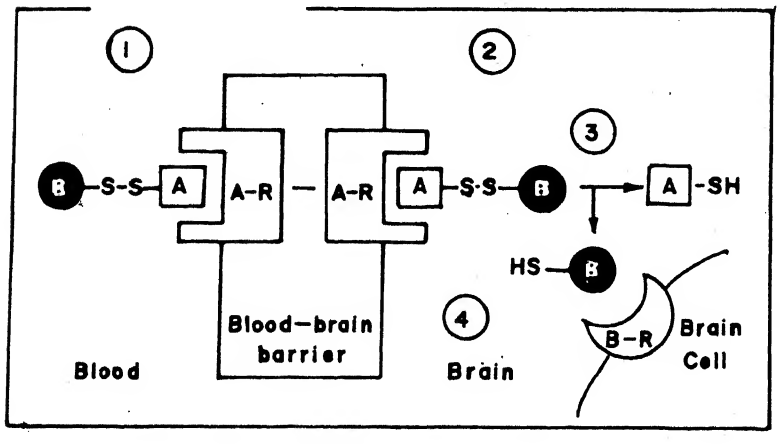
The existing strategies for brain drug delivery fall into 3 general protocols: (1) neurosurgery based, (2) pharmacology based [for small molecules], and (3) physiology based.

Neurosurgery based strategies include intraventricular drug infusion cell therapy or disruption of BBB via a hole in the head. The pharmacology based strategies for delivery of small molecules through BBB include lipidization strategies and liposomes. Thus, acetylation of a single hydroxyl group increases permeability by a factor of ten or more. The lipid soluble molecules are thought to be transported through the barrier by accessing small pores. Composites having a molecular weight more than 600 cannot access this path.

A more effective strategy is to modify the drug so that the compound has a profile that mimics a nutrient which has access to carrier mediated transport at the barrier. For example, dopamine is poorly transported through the BBB. L-Dopa can, because it can take advantage of the amino acid transporter system within the barrier. Similarly adenosine based drugs can take advantage of the adenosine transport.

A "piggy back" ride for a drug through the BBB can be arranged if a non-transporter can be tethered reversibly, to a transporter that has access to BBB, either by absorptive mediated [AM] or receptor mediated [RM] pathway. The model vector for AM is cationized albumin and for RM, OX26 monoclonal antibody (mAb), which is recognized by the transferin receptor [Chart 6.2].

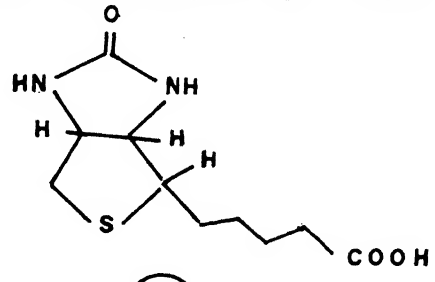
Chart 6.2



B= non transporter (eg. endorphin)
S-S = reversible linker
A = vector (AM/RM)
A-R = vector A receptor
B-R = receptor for drug in the brain

Crucial to the success of the above strategy is the effective scission of the S-S linker in the brain, which is poorly accomplished in many cases. This problem has been solved by introducing avidin-biotin technology into the drug delivery protocols.

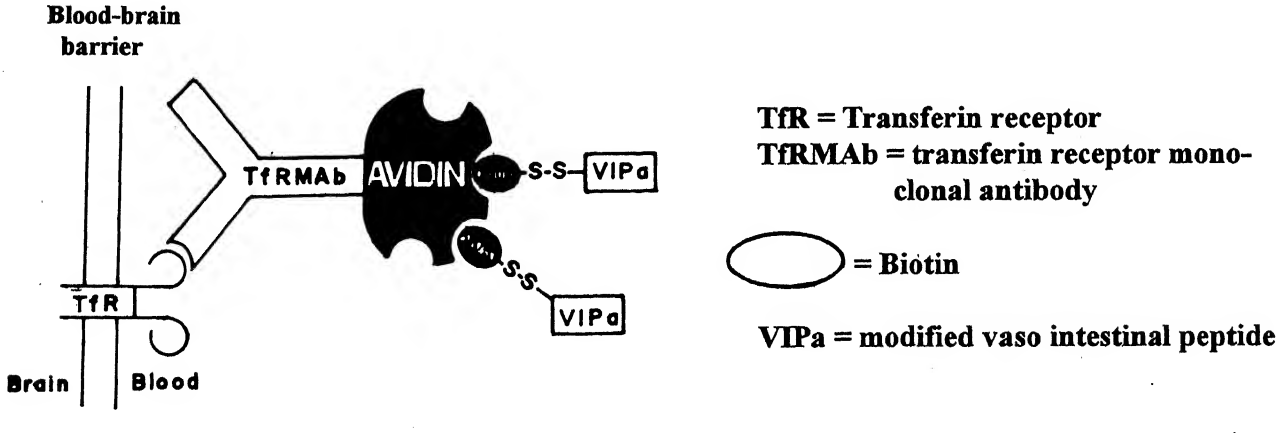
Avidin is a glycoprotein containing 4 essentially identical subunits of ~ 128 amino acids each, where the carbohydrate is attached to Arg17. Biotin has structure (1):



(1)

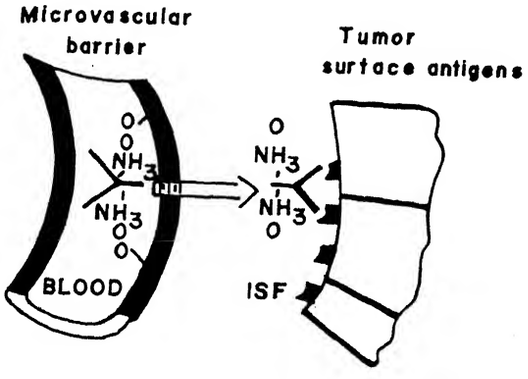
In this approach, a conjugate of avidin and the transport vector (e.g., TfR Mab; Chart 6.3) is prepared which functions as a universal transport vector for drugs into the brain for transport of diverse biotinylated substances across BBB. Peptides and antisense nucleotides can be biotinylated with biotin or analogs that conjugate with either the amino or carboxyl of the drug and here, the biotin-drug linkage can be the disulfide bond, since disulfide reductase, which are abundant in the brain can easily cleave the drug from the avidin vector conjugate, in these cases. Because of the very high affinity of biotin for avidin, $K_d = 10^{-20}$ M, $t_{1/2}$ = 90 days, the biotin of the biotin drug composite practically irreversibly binds to the avidin-vector conjugate. This protocol is illustrated in Chart 6.3 ,with the drug as active analog of vaso intestinal peptide (VIPa).

Chart 6.3



Enhanced antibody delivery to brain tumors through the BBB by "cationization" of the antibody and AM transcytosis through the endothelium is emerging as a powerful tool (Chart 6.4). Cationized antibodies are formed when the isoelectric point of the antibody is raised from the neutral range to the highly alkaline range. Hexamethylenediamine is a useful reagent to accomplish this.

Chart 6.4

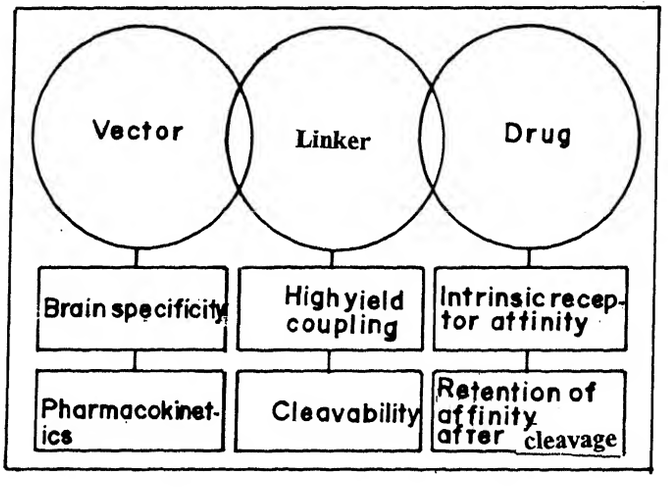


ISF = brain interstitial fluid

The development of systems that can deliver the drug in the brain, lags far behind our understanding of processes and metabolic pathways in the brain. In this area, drug development and drug delivery should go hand in hand. Treatment of brain disorders, an area of great current interest, can make progress only if diverse delivery systems are discovered.

The asymmetric efforts pertaining to drug discovery and drug delivery in this area becomes even more challenging when it is considered how difficult the development of a delivery system is. As an example, crafting of delivery systems for peptides (Chart 6.2) can be analysed (Chart 6.5).

Chart 6.5



A new program for discovering vectors with higher degree of brain specificity is needed. The pharmacokinetics of the vector are crucial because the amount of drug delivered to the brain is a function of, efficiency with which it traverses the BBB and the average plasma concentration of the vector. The linker here plays a crucial role, on the one hand it should be efficiently coupled to the vector and should be amenable to ready cleavage on the other. Finally the drug must have a high intrinsic receptor affinity.

Treatment of disorders of the spinal chord and brain will receive increasing priority in the coming years. The integration of physiology, pharmacokinetics, molecular biology and organic chemistry that is taking place at a rapid pace will provide knowledge to achieve these objectives.

Leading References

1. New approaches to drug delivery through the blood-brain barrier. W.M. Pardridge. Tibtech, **12**, 239 (1994).